Beyond Skin Aging, Towards Health

Selma Mekić

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Promotor

Prof. dr. T.E.C. Nijsten

Overige leden

Prof. dr. M. Kayser

Prof. dr. J.C. Kiefte-de Jong

Prof. dr. E.P. Prens

Copromotoren

Dr. L.C. Jacobs

Dr. L.M. Pardo Cortes

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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?

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Part II

TELANGIECTASIA

CHAPTER 2.1

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

S. Mekić*
M. A. Hamer*
C. Wigmann
D. A. Gunn
M. Kayser
L. C. Jacobs
T. Schikowski
T. Nijsten
L. M. Pardo

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** Shared first authorship*

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

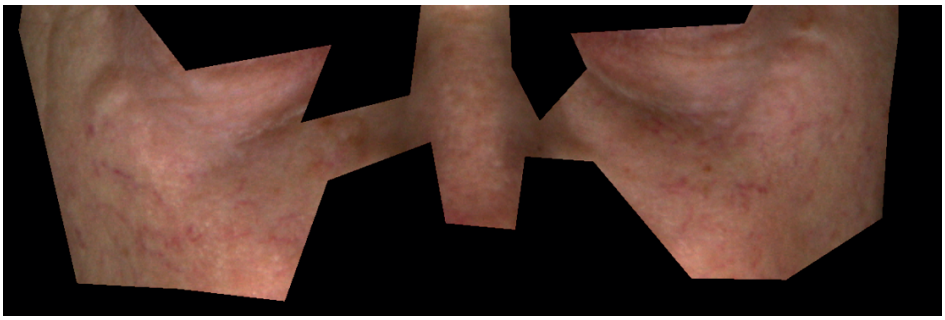
The Rotterdam Study (RS) is an ongoing prospective population-based cohort study of middle-aged to elderly (≥45 years of age) inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands. Since 2010, skin examinations have been conducted by trained physicians, focusing on the most common skin diseases. 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

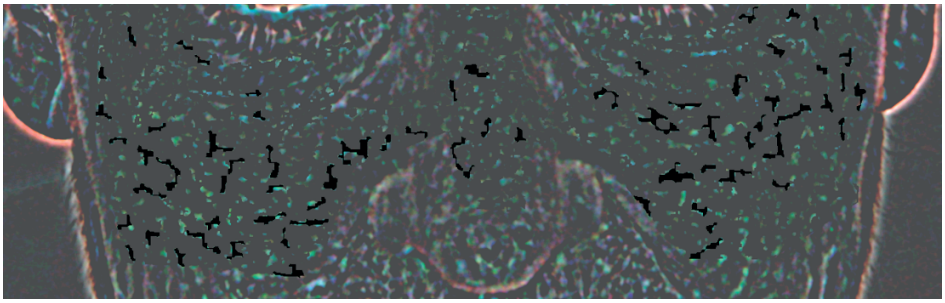


Figure 1 (**a**). Example of masked image. (**b**) Example of image analysis technique where the black structures are picked up as telangiectasia.

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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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).

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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)

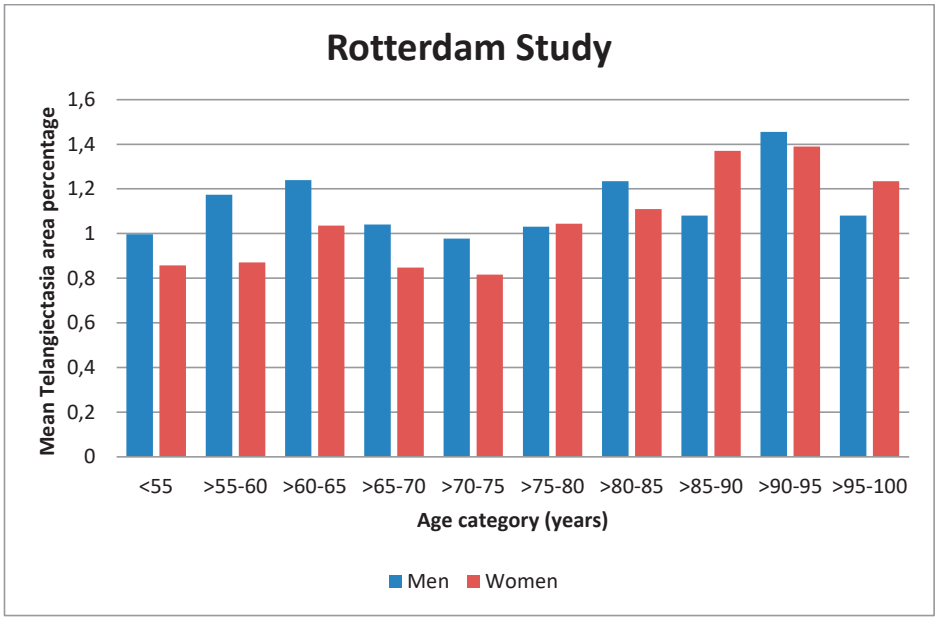
Abbreviations: BMI, body mass index; SD, standard deviation.

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

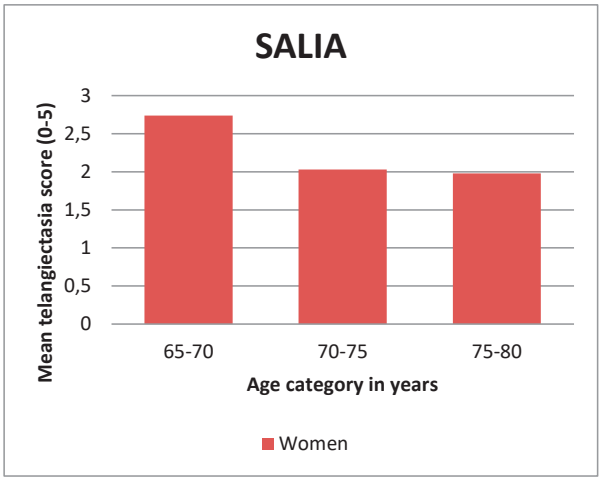
Determinant	ß	95% CI	P-value
Age (per year)	-0.09	**[-0.12, -0.05]**	**<0.001**
BMI (per point)	0.005	[-0.02, 0.03]	0.714
Skin type (Fitzpatrick)			
III/IV	ref	ref	ref
I/II	0.44	**[0.22, 0.66]**	**<0.001**
Regular use of sun protection cream			
No	ref	ref	ref
Yes	-0.21	[-0.45, 0.02]	0.074
Tanning bed 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

S. Mekić
C. Wigmann
D. A. Gunn
L. C. Jacobs
M. Kayser
T. Schikowski
T. Nijsten
L. M. Pardo

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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)].

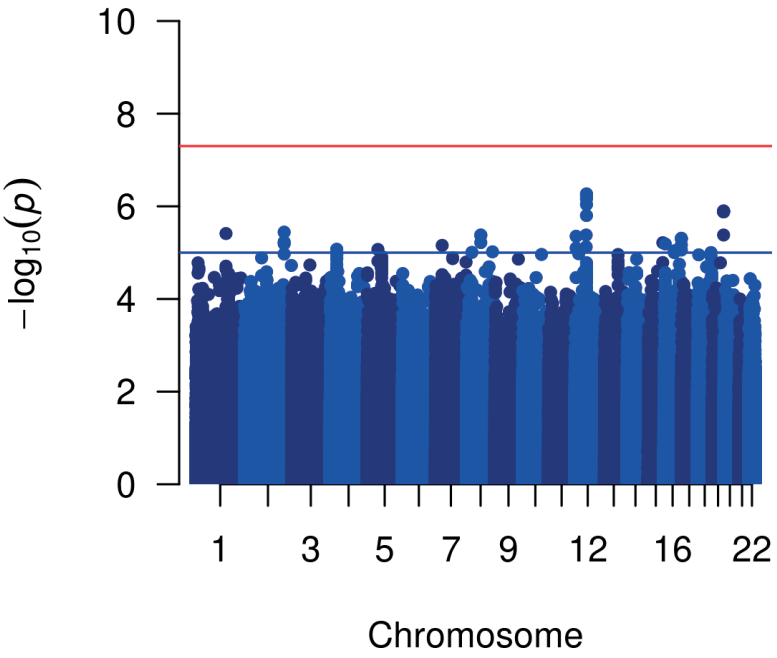

Figure 1. Manhattan plot representing the association between the single nucleotide polymorphisms (SNPs) and the ln-transformed percentage of the face, which is covered with telangiectasia for 2842 men and women. On the x-axis the chromosomes are plotted with each dot representing a SNP on corresponding 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.

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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

S. Mekić
L. C. Jacobs
D. A. Gunn
A. E. Mayes
M.A. Ikram
L. M. Pardo
T. Nijsten

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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.

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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

S. Mekić
D. A. Gunn
L. C. Jacobs
D. J. Hijnen
M.A. Ikram
A. E. Mayes
T. Nijsten
L. M. Pardo

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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

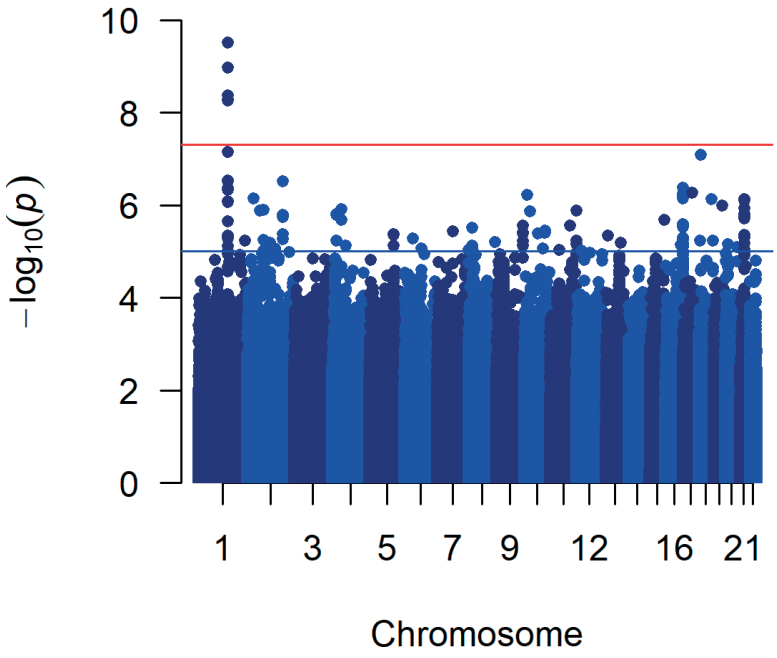

Figure 1. Figure 1. Manhattan plot of GWAS of generalized dry skin. Manhattan plot representing the association between the SNPs and having a generalized dry skin for 530 cases and 1,850 controls. On the x-axis, the chromosomes are plotted with each dot representing an SNP on corresponding chromosomal 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.

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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).

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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

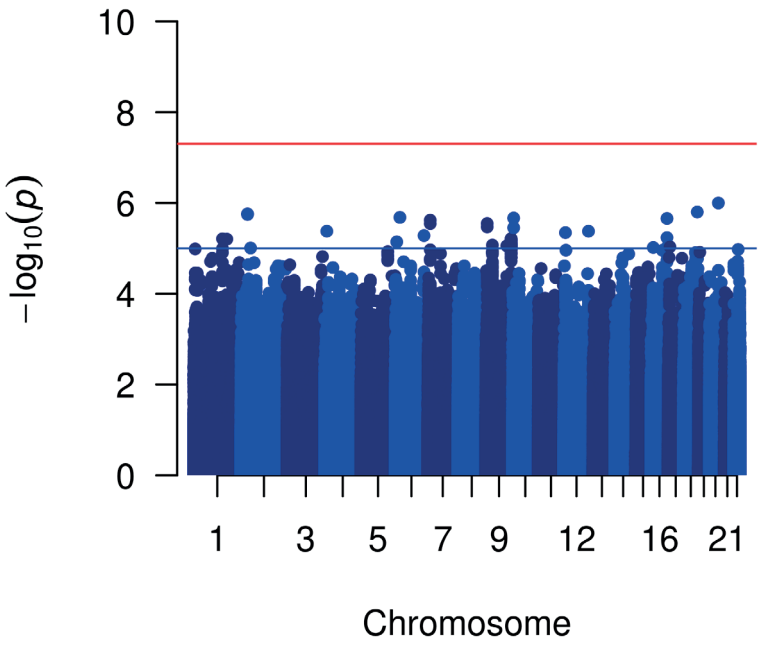
For the highly suggestive SNPs on chromosome 1 (p-value< 5.0*10^{-7}), SNP ID according to RS number, Pvalue = p-value of the association of the GWAS generalized dry skin, NTAG represents the number of SNPs in Linkage Disequilibrium (LD), LEFT and RIGHT present the left and right border of the area of 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

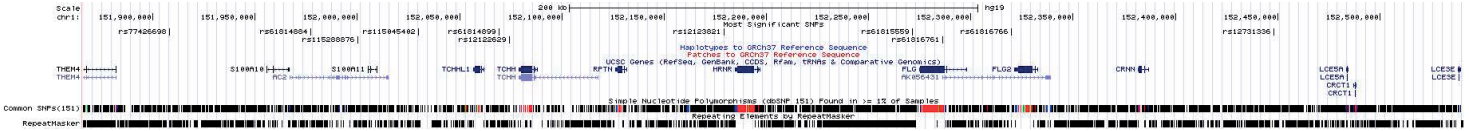
SNP	Gene	LD	P-value GWAS generalized dry skin	P-value GWAS generalized dry skin adjusting for top hit	P-value GWAS generalized dry skin adjusting for *FLG* SNPs
rs12123821	*HRNR*	NOT IN REGION	3.05E-10	x	3.26E-10
rs115045402	*AC2*	NOT IN REGION	1.06E-09	n.s.	1.24E-09
rs115288876	*AC2*	REGION 1	4.24E-09	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

S. Mekić
L. C. Jacobs
M.A. Ikram
J.D. Schoufour
D. A. Gunn
J.C. Kiefte- de Jong
T. Nijsten
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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].

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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.

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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.

CHAPTER 4.2

Youthful facial looks decrease the likelihood of several age-related morbidities in the middle-aged to elderly: a cross-sectional study

S. Mekić
L.M. Pardo
D. A. Gunn
L. C. Jacobs
M. A. Hamer
M. A. Ikram
E. J. Vinke
M. W. Vernooij
A. E. G. Haarman
E. F. Thee
J. E. Vergroesen
C. C. W. Klaver
P.H. Croll
A. Goedegebure
K. Trajanoska
F. Rivadeneira
J. B. J. van Meurs
B. Arshi
M. Kavousi
E. W. de Roos
G. G. O. Brusselle
M. Kayser
T. Nijsten
Submitted

ABSTRACT

Importance: Looking older for one's chronological age is associated with a higher mortality rate. Yet it remains unclear how perceived facial age relates to morbidity and the degree to which facial aging reflects systemic aging of the human body.

Objective: To investigate the association between delta perceived age (ΔPA) and the most prevalent age-related morbidities of different organ systems, where ΔPA represents the difference between perceived age (PA) and chronological age.

Design: Cross-sectional analysis of data from the Rotterdam Study

Setting: Population-based cohort study in the Netherlands

Participants: 2,679 middle-aged to elderly men and women from European descent

Exposure: PA was divided in 5-year categories using high resolution facial photographs by a panel of men and women who were blinded for chronological age and medical history. A linear mixed model was used to generate the mean perceived ages. The delta between the mean perceived age and chronological age was calculated (ΔPA), where a higher (positive) ΔPA means the subjects looks younger for his age and a lower (negative) ΔPA that the person looks older.

Main outcomes and measures: ΔPA was tested as a continuous variable for association with presence of the most prevalent and characteristic age-related cardiovascular, pulmonary, ophthalmological, neurocognitive, renal, skeletal and auditory morbidities in separate regression analyses, adjusted for age and sex (model 1) and additionally for body mass index, smoking and sun exposure (model 2). We observed a higher ΔPA (i.e., looking younger for age) associated with less osteoporosis, less COPD, less age related hearing loss, less cataracts, while with better global cognitive functioning.

Conclusions and relevance: Looking younger for one's age is associated with less systemic morbidity and better cognitive function, suggesting that both physical and cognitive health are externally visible in the human face. Overall, our study underlines perceived facial age as a biomarker for healthy aging in northwestern Europeans.

INTRODUCTION

Since ancient history, mankind has been in a quest for the source of eternal youth. As global longevity keeps increasing, this desire to preserve youthful looks might be more relevant today than ever before. However, maintaining a youthful appearance does not merely serve esthetic purposes as it also associates with better psychosocial wellbeing [1]. In addition, perceived age (PA), or how old a person is estimated to look, has been linked to parameters of health and morbidity. Looking older for one's age even associates with mortality. In a monozygotic twin study where the older looking twin died first, the PA difference was on average 1.4 years [2]. Survival analyses showed that PA is a stronger predictor for mortality than chronological age, suggesting that PA could be a clinical biomarker. In a Japanese study of 273 men and women, looking younger for one's age significantly associated with lower carotid intima media thickness [3]. Facial wrinkles, graying of the hair and baldness correlated with myocardial infarction in a study of 20,000 men of the Copenhagen City Heart Study [4]. A Dutch study of 463 women aged 25-93 found that higher PA was significantly associated with a lower bone mineral density (BMD) [5].

Aforementioned studies have mostly focused on subgroups of the population and investigated selected or single organ age-related morbidities, and none have tried to replicate previous findings. Understanding how PA relates to aging of multiple organ systems requires exploration of multi-organ morbidities in a large sample of men and women and correction for chronological age should be performed.

In this cross sectional study, we aimed to investigate the association between ΔPA, the difference between chronological age and perceived age indicating how old one looks given their chronological age, and the most prevalent age-associated cardiovascular, pulmonary, ophthalmological, neurocognitive, renal, skeletal and auditory morbidities in a sample of the population-based Rotterdam Study of 2,679 middle-aged to elderly men and women.

MATERIALS AND METHODS

Study population

Subjects were selected from the Rotterdam Study, an ongoing large population-based cohort study, situated in a suburb of Rotterdam, the Netherlands. 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. Extensive details and objectives have been described elsewhere [6]. Dermatological screening was incorporated from 2010 onwards. Between September 2010 and July 2014, 2,679 men and women were photographed for whom PA was assessed.

Phenotyping

During dermatological examination at the research center, high-resolution facial images were taken using the Premier 3dMD face 3-plus UHD camera (3dMD, Atlanta, Georgia, USA). Participants were instructed not to wear any creams, make-up or jewelry. Using standardized 2D facial images from the 3dMD system, PA was assessed by a panel of independent observers who were blinded to chronological age. For this, a validated method was applied which previously has been explained in detail [7]. In short; assessors (Unilever employees of various ages and sex) selected a five-year age range they thought the subject looked using a frontal and side image, with the middle age taken as the assessed age. Intra-rater variability was r=0.65 which was similar to that reported in other studies (r=0.67) [8]. A linear mixed model with subject and assessor as random effects and order of assessment as a fixed effect was used to generate the mean perceived ages. Each image was scored by on average 27 assessors.

Subsequently, ΔPA was calculated by subtracting PA from chronological age, e.g. a ΔPA of 7 means one looks 7 years younger than their chronological age. The interaction term ΔPA x sex was tested but not significant in several regression analyses, hence men and women were analyzed together.

Variables and selection and definitions of morbidity

Chronological age and sex were collected from the database. Smoking status and number of pack-years were reviewed from general interviews. Body Mass Index (BMI) was calculated as weight in kg/ (height in meter^2). Information on ultraviolet (UV) exposure was collected via questionnaire. Four UV parameters were available for 95% of our population. These were binary variables and accounted for easily getting sunburned, overwintering in a sunny country during winter during the last 5 years, living in a sunny country >1 year and wearing sunglasses or a hat as sun protection measures. These variables were described in detail previously [9] and have been used in other analyses [9, 10].

We aimed to include up to 3 most prevalent and characteristic age-related diseases of each organ system which is studied in the Rotterdam Study. Conditions that are primarily lifestyle driven such as diabetes mellitus type 2 and liver cirrhosis were not included.

Renal impairment was characterized as having a Glomerular Filtration Rate (GFR) <60 ml/min. Atrial fibrillation was diagnosed using three methods (including repeated electrocardiograms) that have been described in detail before [11]. Ischemic cardiovascular disease (CVD) was defined as history of myocardial infarction, revascularization or both. Chronic Obstructive Pulmonic Disease (COPD) was diagnosed with an algorithm based on spirometry reports, files from general practitioners and hospital discharge letters [12]. Osteoarthritis was radiographically scored and defined as Kellgren-Lawrence (KL) score ≥2 for either knee, hip or both [13]. BMD was measured at the femoral neck and lumbar spine. Osteoporosis was present when BMD T-score was <-2.5 [14]. Age-related Macular Degeneration (AMD) was assessed using fundus photographs according to the modified international classification and grading system in stages 0 (no signs

of AMD or only small hard drusen) up to stage 4 (late AMD) [15]. Presence of AMD included stages 1-4. Cataract was scored as present using pseudophakia cases as proxy, i.e. the natural lens had been replaced with an intraocular artificial lens. Glaucoma was scored as present if glaucomatous visual field loss (GVFL) was present [16]. Age-related hearing loss (ARHL), also known as presbycusis, was measured in decibel (dB) and described before in the Rotterdam Study [17]. In this study we defined ARHL as high frequency hearing loss (2-8 kHz) since increased hearing thresholds in these frequencies are most characteristic for presbycusis. Because there were few cases with severe hearing loss and loss of a few decibel can already be of significant clinical importance, ARHL was investigated continuously. Participants with conductive hearing loss (airbone gap >15 dB) were excluded from this analysis. To assess neurocognitive outcomes we did not use dementia cases because of their selection bias in presentation at the research center, instead we used a measure of cognitive impairment as continuous proxy. The g-factor, which accounted for 49,2% of the population variation in cognition, here was used to measure global cognition [18].

Statistical analysis

To assess the association between ΔPA and the different morbidities, separate regressions were performed with the age-related morbidity as outcome. Most outcomes were dichotomous thus logistic regression was predominantly performed which provided an odds ratio (OR) for ΔPA per year, which was subsequently calculated per five years. For ARHL and g-factor, a linear regression model was considered. Assumptions for both logistic and linear regression models were met. Correction for multiple testing was not performed based on the basis premises of empirical research [19] and p-value <0.05 was considered significant.

ΔPA was tested as independent continuous variable in the model, allowing to detect small effect sizes. Two models were applied for all conditions, namely a sex and age adjusted model (model 1) and a model which was adjusted for sex, age, BMI, UV exposure, smoking status and number of pack-years (model 2). UV exposure and smoking are well known influencers of both exposure and outcome, BMI although known to influence health and morbidity, is also associated with a less wrinkled appearance [9] probably due to the filler effect of facial fat. To allow for non-linear correction for age, age^2 was tested for association with tested morbidities in all models and added if significant ($p<0.1$). PA was assessed from 4 batches of photographs which showed subtle differences in mean PA, therefore the 'PA batch' variable was added as covariate in all analyses. Individuals with missing data were excluded.

Sensitivity and additional analyses

Cognitive function is strongly linked with education level. Therefore, we additionally adjusted the regression with g-factor as outcome for education level. This was performed by correcting for two dummy variables in the linear regression, of three education levels (low/ medium/ high) which have previously been described and used in skin aging analyses of the Rotterdam

Study [10]. Also, since smoking and COPD are strongly linked, a sensitivity analysis in never smokers was performed for COPD.

To better understand the etiology of the significant associations between ΔPA and age-related diseases and which skin aging phenotypes might be driving the associations, we additionally replaced ΔPA by wrinkle %, telangiectasia % or pigmented spot % of the face. These measures were semi-automatically obtained using the same frontal facial photographs, which has been described in detail previously [9, 20, 21].

RESULTS

Population characteristics

Our study population had a median age of 65.8 years skewed to higher ages and consisted of a slightly higher percentage of women (54.1%) than men (45.9%) (Table 1). A higher ΔPA means the person looks younger for his age and a lower PA Δ that the person looks older. To illustrate descriptive characteristics between individuals who look young or old for their chronological age, the cohort was divided into tertiles based on ΔPA and categorized in 'looks younger', 'looks their age' and 'looks older'. The younger looking group was estimated on aver-

Table 1. Characteristics of tertiles of ΔPA

Variable	1st N=893 Looks younger	2nd N=893 Looks their age	3rd N= 893 Looks older	Total N=2679
ΔPA [1] – median (IQR)	5.3 (4.1)	0.1 (2.4)	-5.6 (4.7)	0.1 (7.5)
Perceived age - median (IQR)	62.5 (8.8)	64.1 (9.9)	70.8 (9.5)	65.7 (10.9)
Age – median (IQR)	68.8 (9.3)	64.3 (9.4)	64.7 (8.1)	65.8 (9.9)
Sex – N (%)				
Male	546 (61.1)	401 (44.9)	284 (31.8)	1231 (45.9)
Female	347 (38.9)	492 (55.1)	609 (68.2)	1448 (54.1)
Smoking status – N (%)				
Never	297 (33.3)	292 (32.7)	228 (25.5)	817 (30.5)
Former	497 (55.7)	455 (51.0)	403 (45.1)	1355 (50.6)
Current	97 (10.9)	146 (16.3)	260 (29.1)	503 (18.8)
Missing	2 (0.2)	0 (0.0)	2 (0.2)	4 (0.1)
Pack-years[2] – median (IQR)	3.3 (20.5)	3.3 (18.8)	9.0 (27.4)	5.0 (22.4)
Missing - N (%)	4 (0.1)	0 (0.0)	0 (0.0)	4 (0.1)
BMI[3] – mean (SD)	28.1 (4.1)	28.0 (4.5)	26.9 (4.3)	27.6 (4.4)

** Due to rounding the total might be slightly above or below 100%. [1] Chronological age – perceived age in years e.g. ΔPA 6.08 years means subject looks on average 6.08 years younger than their chronological age; [2] Number of pack-years smoking cigarettes, cigars or pipes, where one pack-year equals smoking 20 cigarettes every day during one year; [3] Body Mass Index in $kg/meter^2$

age 5 years younger than their chronological age and was predominantly male (61%), less often a smoker and had the highest BMI. Distributions of the investigated morbidities are presented in Supplementary Table S1.

ΔPA association with comorbidities

Looking younger for one's age did not significantly associate with atrial fibrillation, renal impairment nor ischemic CVD (Table 2). However, youthful looks did significantly link with a lower risk of COPD, even after adjusting for smoking status and pack-years (OR, 0.85; 95%CI, 0.77; 0.95). Furthermore, looking five years younger for one's age reduced the likelihood of having osteoporosis by a quarter when adjusted for confounders (OR, 0.76; 95%CI 0.62; 0.93) whereas no link between youthful looks and osteoarthritis was found. Of the ophthalmological conditions tested, youthful looks significantly linked with lower risk of cataracts even when corrected for confounders (OR, 0.84; 95%CI 0.73; 0.97), while not with AMD nor with GVFL. Youthful looks also significantly associated with lower risk of ARHL (model 2; B, -0.76; 95%CI, -1.35; -0.17) and with a higher cognitive function (g-factor; model 2; B, 0.07; 95%CI, 0.04; 0.10) in both models.

Sensitivity analyses

Additionally, correcting the g-factor regression for education level slightly decreased the beta, although results remained significant for both model 1 (B, 0.07; 95%CI, 0.04; 0.09) and model 2

Table 2. Association ΔPA (continuous) with age-related morbidities

	N	Model 1*		Model 2*	
Outcome		**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Renal impairment	2374	1.01 (0.88, 1.15)	0.917	1.00 (0.87, 1.15)	0.999
Atrial fibrillation	2458	1.12 (0.92, 1.35)	0.256	1.12 (0.92, 1.37)	0.263
Ischemic CVD	2458	0.93 (0.80, 1.08)	0.344	0.94 (0.80, 1.10)	0.438
COPD	2470	**0.71 (0.64, 0.78)**	**<0.001**	**0.85 (0.77, 0.95)**	**0.004**
Osteoarthritis	2010	1.00 (0.91, 1.10)	0.963	0.97 (0.88, 1.07)	0.586
Osteoporosis	2377	**0.64 (0.52, 0.77)**	**<0.001**	**0.76 (0.62, 0.93)**	**0.008**
AMD	2470	0.96 (0.88, 1.03)	0.239	0.97 (0.90, 1.05)	0.467
Cataract	2366	**0.85 (0.74, 0.97)**	**0.016**	**0.84 (0.73, 0.97)**	**0.014**
GVFL	2382	0.95 (0.70, 1.29)	0.728	0.92 (0.67, 1.26)	0.598
Outcome		**B (95%CI)**	**p-value**	**B (95%CI)**	**p-value**
G-factor	2021	**0.09 (0.06, 0.12)**	**<0.001**	**0.07 (0.04, 0.10)**	**<0.001**
ARHL**	2110	**-0.81 (-1.38, -0.25)**	**0.005**	**-0.76 (-1.35, -0.17)**	**0.012**

* The OR and Beta are presented **per 5 years** looking younger. Model 1; chronological age, batch and sex adjusted. Model 2; chronological age, batch, sex, UV, smoking status, pack-years and BMI adjusted. Age^2 term was significant (p-value <0.1) and therefore added to ARHL (model 1&2), G-factor (model 1&2), cataract (model 1&2) and osteoporosis (model 1); ** Participants with an air-bone gap of 15 dB or more were excluded to eliminate conductive hearing loss. Significant associations (p-value<0.05) are presented in **bold**

(B, 0.05; 95%CI, 0.04; 0.10). Even in never smokers (n=760) looking five years younger for one's age was significantly associated with a quarter less COPD (model 1 OR, 0.73; 95%CI, 0.57-0.94; model 2 OR, 0.75; 95%CI, 0.58-0.97).

Replacing ΔPA by other skin aging phenotypes (wrinkles, pigmented spots or telangiectasia) revealed that all significant associations between ΔPA and age-related diseases, bar ARHL, were significantly driven by facial wrinkle percentage. Pigmented spots and telangiectasia were not associated with any of the investigated morbidities (Supplementary Table S2).

DISCUSSION

In this cross-sectional study, youthful looks associated with reduced likelihood of several well-known age-related morbidities of the middle-aged to elderly. In particular, youthful looks significantly associated with lower prevalence of osteoporosis, COPD, cataract, and ARHL. In addition, looking younger for one's age significantly associated with higher global cognition. These findings indicate that physical as well as cognitive health measures are associated with young looks. Our findings underline perceived facial age as a useful biomarker of healthy aging and imply that ΔPA may serve as an additional clinical sign in the diagnostic work-up of physicians.

Through which physiological pathways and molecular mechanisms ΔPA affects health and pathogenesis of different diseases is not clear yet, although it appears to commence earlier in life. A study in 954 young adults showed that faster aging individuals felt less healthy and were rated as looking older by independent observers [22]. We showed that ΔPA was driven by facial wrinkle percentage. This was not surprising since these two phenotypes have shown to cluster before as opposed to pigmented spots and telangiectasia, which are more skin color associated [23]. Decreased cellularity, hence, could be hypothesized one of the explanatory mechanisms of the found associations. A decrease in cutaneous fibroblasts and in the production of collagen causes a more aged look [24], whereas a decrease in osteoblast activity accounts for less bone density [25]. In cell culture, fibroblasts are nearly identical to osteoblasts and all genes expressed in fibroblasts are also expressed in osteoblasts [26]. Fibroblast growth factors (FGFs) show to play an important role in skin aging [27] but they target many more tissues, e.g. the disruption of the *FGF-2* gene results in decreased bone mass and bone formation [28]. Hence, communalities in cell properties as well in signaling pathways might be at the basis of the link between youthful looks and bone density. In addition, osteoporosis might be associated with facial bone loss causing a more aged look as longitudinal facial imaging studies show bone loss and patterns of remodeling in aging [29]. Dental panoramic radiographs show that 1 mm decrease in mandibular cortical width increases the likelihood of osteopenia or osteoporosis to 47% [30]. The link between perceived facial age and osteoporosis has been established before in a sample of 460 Dutch women, even when adjusted for BMI, hormone replacement therapy and menopause [5],

highlighting the robustness of this link. Etiology of age-related cataracts is multifactorial and includes breakdown and aggregation of protein, damage to fiber cell membranes and changed ion transport due to lens dehydration [31]. Three main types of age-related cataracts have different mechanism but share risk factors such as increasing age, cigarette smoking and UV-light [32], which are also strong predictors of PA and wrinkling [9]. Although we attempted to adjust for smoking status and UV-related parameters in our analyses other unknown factors could also play a role in the PA and cataract association.

Some morbidities have strong life-style related confounders, although we tried to correct for them and minimize residual confounding. An example is COPD, which is very strongly associated with smoking [33]. However, a sensitivity analysis showed a significant link even in never smokers suggesting mechanisms or confounders other than smoking are involved in the link between ΔPA and COPD. Non-tobacco related COPD includes other causes such as air pollution, occupational exposures (e.g. farming) and poor nutrition [34], which also have been linked with skin aging [10, 35]. ARHL also has many lifestyle-related determinants [17] however, in our analysis we did not correct for one of the most vital one, noise exposure, due to lack of data. Nevertheless, this might capture the link between youthful looks and less ARHL as less outdoor work likely means less occupational UV and noise exposure.

Cellular senescence is probably the key process at the basis of most associations between the significant morbidities and ΔPA. Telomerase length in blood, often considered a biological measure of cellular senescence, also associates with PA in a twin study, where looking older was significantly associated with shorter telomeres in 279 men and women [36]. Furthermore, senescent melanocytes associate with PA and greater facial wrinkling highlighting a direct link between senescence in skin and perceived age [37]. In the aforementioned twin study, looking older for one's age was also significantly associated with lower parameters of cognition measured by both the MMSE and the g-factor, which was replicated in the current study. Whereas, the first genetic study into PA showed that the effect of certain *MC1R* gene variants resulted in looking up to 2 years older of age, revealing pigmentation genes also play a part in PA mechanisms [7].

Despite a number of conditions associating with ΔPA, many of our investigated morbidities were not associated with ΔPA. We hypothesize that some of these are more lifestyle driven, featuring a more prominent inflammatory pathway like ischemic CVD, or degenerative like osteoarthritis, than that they are driven by cell senescence. Others such as AMD are multifactorial and are also known to be driven by poor diet [38]. In addition, lack of power could also be an explanation for not finding significant associations e.g. for glaucoma and renal failure.

We investigated a large population-based sample of men and women and aimed to investigate the most characteristic and prevalent age-related morbidities of the elderly. However, collider bias in study population and selection bias in investigated morbidities cannot be excluded having influenced our findings. Nevertheless, discovering multiple significant associations highlights the internal validity of our data. Moreover, in this study we subsequently adjusted our regression analyses for age and age^2, because a ΔPA of 5 years might have a different clinical

meaning when aged 55 versus when aged 80 years while allowing for correction of non-linear age effects.

Reliability and heterogeneity of the scoring panel can influence ΔPA. In this study, ΔPA was scored by a large panel of UK men and women of various ages with a high number of average scorings, to maximize validity. However, a validation study showed that subject age and assessor sex, nationality, age and aging expertise had little effect on the generated PA data [8]. Investigating the ΔPA as a continuous variable as opposed to categories of youthful looks provided smaller effect sizes, however this permitted for greater power in our analyses. Health conscious behavior and reverse causation could have influenced our results, where people who look younger for their age may adhere to a healthier lifestyle resulting in less morbidity. Our results were obtained from a predominantly northwestern European population and generalizability to other populations needs to be investigated in the future. Finally, the design of our study impedes from making causal inferences.

In conclusion, we found that looking younger for one's age is associated with less morbidity of different organ systems as well as better cognitive function. Overall our study demonstrates that both physical and cognitive health are externally visible in the human face, underlining ΔPA as biomarker for healthy aging and indicating that how old someone looks may be used as an additional clinical sign in physical assessment. Future studies should focus on the physiological pathways and molecular mechanisms that may provide causal explanations for our association findings while expanding their scope to different populations. ΔPA might serve as an accessible study model to understanding commonality in aging pathways for different organ systems.

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SUPPLEMENTARY TABLES

Supplementary Table S1. Descriptive statistics of main morbidities

Morbidity	1st N=893 Looks younger	2nd N=893 Looks their age	3rd N= 893 Looks older	Total N=2679
Renal impairment[1] – N (%)				
Yes	123 (13.8)	74 (8.3)	73 (8.2)	270 (10.1)
No	735 (82.3)	781 (87.5)	784 (87.8)	2300 (85.9)
Missing	35 (3.9)	38 (4.3)	36 (4.0)	109 (4.1)
Atrial Fibrillation[2] – N (%)				
Yes	50 (5.6)	41 (4.6)	23 (2.6)	114 (4.3)
No	835 (93.5)	849 (95.1)	869 (97.3)	2553 (95.3)
Missing	8 (0.9)	3 (0.3)	1 (0.1)	12 (0.4)
Ischemic CVD[3] - N (%)				
Yes	101 (11.3)	48 (5.4)	50 (5.6)	199 (7.4)
No	784 (87.8)	842 (94.3)	842 (94.3)	2468 (92.1)
Missing	8 (0.9)	3 (0.3)	1 (0.1)	12 (0.4)
COPD[4] - N (%)				
Yes	155 (17.4)	121 (13.5)	182 (20.4)	458 (17.1)
No	738 (82.6)	772 (86.5)	711 (79.6)	2221 (82.9)
Osteoarthritis[5] - N (%)				
Yes	256 (28.7)	190 (21.3)	189 (21.2)	635 (23.7)
No	517 (57.9)	513 (57.4)	538 (60.2)	1568 (58.5)
Missing	120 (13.4)	190 (21.3)	166 (18.6)	476 (17.8)
Osteoporosis[6] - N (%)				
Yes	25 (2.8)	25 (2.8)	45 (5.0)	95 (3.5)
No, osteopenia	433 (48.5)	439 (49.2)	483 (54.1)	1355 (50.6)
No, normal BMD	399 (44.7)	392 (43.9)	333 (37.3)	1124 (42.0)
Missing	36 (4.0)	37 (4.1)	32 (3.6)	105 (4.0)
AMD[7]- N (%)				
Yes	429 (48.0)	309 (34.6)	331 (37.1)	1069 (39.9)
No	464 (52.0)	584 (65.4)	562 (62.9)	1610 (60.1)
Cataract[8] - N (%)				
Yes	114 (12.8)	69 (7.7)	78 (8.7)	261 (9.7)
No	749 (83.9)	780 (87.3)	776 (86.9)	2305 (86.0)
Missing	30 (3.4)	44 (4.9)	39 (4.4)	113 (4.2)
GVFL[9] - N (%)				
Yes	20 (2.2)	9 (1.0)	14 (1.6)	43 (1.6)
No	817 (91.5)	844 (94.5)	839 (94.0)	2500 (93.3)
Missing	56 (6.3)	40 (4.5)	40 (4.5)	136 (5.1)

Supplementary Table S1. Descriptive statistics of main morbidities (continued)

Morbidity	1st N=893 Looks younger	2nd N=893 Looks their age	3rd N= 893 Looks older	Total N=2679
Cognitive funtion[10]				
G-factor – mean (SD)	0.04 (0.93)	0.38 (0.85)	0.34 (0.91)	0.25 (0.91)
Missing – N (%)	163 (18.3)	164 (18.4)	165 (18.5)	492 (18.4)
ARHL[11]				
HFHL in dB – mean (SD)	35.9 (17.1)	29.0 (16.3)	29.4 (16.4)	31.3 (17.1)
Excluded – N (%)	12 (1.3)	10 (1.1)	10 (1.1)	32 (3.6)
Missing – N (%)	187 (20.9)	147 (16.4)	124 (13.9)	458 (17.1)

[1] Glomerular Filtration Rate (GFR) <60 ml/min; [2] Prevalent Atrial Fibrillation diagnosed by validated method; [3] Ischemic Cardio Vascular Disease (CVD); history of either myocardial infarction, revascularization or both; [4] Prevalence of Chronic Obstructive Pulmonary Disease, based on spirometry reports and GP and hospital data; [5] Prevalence of osteoarthritis, defined as Kellgren-Lawrence (KL) score ≥2 for either knee, hip or both; [6] Prevalence of osteoporosis, defined as BMD T-score <2,5; [7] Age-related Macular Degeneration, ranging from early to late AMD; [8] History of cataract, defined as pseudophakia cases; [9] GVFL, defined as presence of glaucomatous visual field loss; [10] Cognitive function, using the g-factor as proxy for cognitive decline and (subclinical) dementia; [11] Age-related hearing loss (ARHL), defined as high frequency (2-8 kHz) hearing loss (HFHL) in decibel (DB) measured on 'best' ear. Subjects with conductive hearing loss are excluded from this analysis.

Supplementary Table S2. Wrinkles, Telangiectasia & Pigmented spots instead of ΔPA

	Wrinkle % (model 2)		**Telangiectasia % (model 2)**		**Pigmented spot % (model 2)**	
Outcome	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
Renal impairment	1.002 (0.945, 1.062)	0.952	0.965 (0.687, 1.354)	0.836	0.869 (0.678, 1.115)	0.270
Atrial fibrillation	0.994 (0.914, 1.080)	0.879	1.048 (0.651, 1.687)	0.848	0.835 (0.572, 1.217)	0.384
Ischemic CVD	0.999 (0.934, 1.069)	0.980	1.047 (0.719, 1.526)	0.809	1.295 (0.969, 1.731)	0.081
COPD	**1.073 (1.025, 1.124)**	**0.003**	1.035 (0.801, 1.336)	0.794	0.969 (0.796, 1.181)	0.758
Osteoarthritis	1.025 (0.980, 1.071)	0.278	0.895 (0.704, 1.136)	0.360	0.927 (0.788, 1.090)	0.359
Osteoporosis	**1.128 (1.038, 1.225)**	**0.004**	1.487 (0.934, 2.368)	0.095	1.182 (0.847, 1.648)	0.325
AMD	0.993 (0.956, 1.031)	0.708	1.107 (0.909, 1.348)	0.314	0.985 (0.859, 1.130)	0.828
Cataract	**1.063 (1.003, 1.125)**	**0.038**	0.814 (0.569, 1.166)	0.262	1.066 (0.839, 1.355)	0.600
GVFL	0.922 (0.794, 1.071)	0.289	1.124 (0.507, 2.492)	0.774	1.279 (0.777, 2.105)	0.333
Outcome	**B (95%CI)**	**p-value**	**B (95%CI)**	**p-value**	**B (95%CI)**	**p-value**
G-factor	**-0.021 (-0.035, -0.007)**	**0.004**	-0.036 (-0.109, 0.037)	0.337	0.021 (-0.030, 0.072)	0.419
ARHL**	-0.016 (-0.283, 0.251)	0.905	0.881 (-0.499, 2.260)	0.211	0.587 (-0.391, 1.565)	0.239

Multivariable regression with age-related disease as outcome. Instead of ΔPA, wrinkle percentage, telangiectasia percentage or pigmented spot % covering the face is used as continuous independent. OR and B are presented per 1% increase in aging phenotype area of the total face area.

Part V

DISCUSSION AND APPENDICES

CHAPTER 5.1

General discussion

DISCOVERIES IN THIS THESIS

In this thesis I investigated the association between genetic and lifestyle determinants, including measures for health and disease with different skin aging phenotypes including telangiectasia, wrinkles, dry skin and perceived age. I learned that although there are differences in risk factors between the different phenotypes, some of them show more overlap than others. More recently the terms hypertrophic skin aging (featuring coarse wrinkling and increased skin thickness) and atrophic skin aging (featuring discoloration and telangiectasia) have been adopted in skin aging research, illustrating an example of the aforementioned overlap [1].

EXPLORATION OF A LESS WELL-KNOWN SKIN AGING PHENOTYPE

Distinct skin aging phenotypes differ in epidemiological and genetic determinants, however the latest research shows that some of them cluster together. A principal component analysis of seven skin aging phenotypes derived from the Rotterdam Study showed three main clusters of skin aging, namely a hypertrophic, atrophic and cancerous component, potentially revealing shared mechanisms and risk factors [2]. Most of the skin aging research focused on this hypertrophic component including wrinkles as explained in **Chapter 1,** but we aimed to expand the scope of skin aging research and also investigate less well-known signs of skin aging. Hence, two studies regarding telangiectasia were included in this thesis, an atrophic phenotype. The first study investigated determinants and lifestyle factors associated with facial telangiectasia (**Chapter 2.1**). Most important variables with largest effects associated with more facial red veins were light skin color type and smoking, as was previously described in other studies [3-5]. Other associated determinants included older age, female sex and a high susceptibility to sunburn in both sexes. Current smokers had more than a third more telangiectasia and former smokers still had 11.6% more telangiectasia compared with never smokers. This suggests that smoking causes irreversible signs of skin aging, as described for several other skin aging phenotypes before [5-8]. Smoking causes vasoconstriction of small vessels leading to a chronic hypoxemic state in the skin [9], which can result in proliferation of new red veins. Furthermore, smoking induces DNA damage, elastosis and atrophy of the skin [10], all of which deteriorate the skins foundation and can make red veins more visible. Smoking was also associated with more generalized dry skin (**Chapter 3.1**), probably due to aforementioned mechanisms. Pale skin color showing more telangiectasia might be because pale skin is more translucent than darker skin. Alternatively, less pigmentation allows for more DNA damage trough UV-induced reactive oxygen species (ROS). In a SNP-based candidate gene approach using known pigmentation genes (**Chapter 2.2**), we found genes *KIAA0930*, *SLCA45A2* and *MC1R* to be significantly associated with a higher telangiectasia percentage, but not independently of skin color. This finding

underlines skin color as one of the most important determinants for telangiectasia found in epidemiological studies.

Microvascular dysfunction is common in patients who suffer from insulin resistance and might explain why we found increasing BMI associated with more telangiectasia in women [11]. This is in contrast with other skin aging phenotypes such as wrinkling and xerosis cutis where a higher BMI associates with fewer wrinkles - probably due to the filler effect of facial fat - and with less dry skin (**Chapter 3.1)**. In addition, individuals with pale skin have fewer wrinkles but more telangiectasia. Overall, this teaches us that although some determinants for skin aging phenotypes overlap (such as smoking and UV-exposure) or cluster, others differ between the distinct skin aging signs, which suggests heterogeneity in skin aging mechanisms and highlights the need for separate analyses.

Although some lifestyle factors have a clear influence on telangiectasia, genetic variants have much smaller effects on these small visible blood vessels. A genome-wide association study (GWAS) was performed using 2842 men and women of northwestern European descent (**Chapter 2.2**). No genome-wide significant variants were found to be associated with facial telangiectasia percentage in our hypothesis-free approach. After all, this was not surprising since the power for detecting such associations was low in our study. Nevertheless, in our dry skin GWAS we did find genome-wide significance, even in a small sample size, underlining that for telangiectasia there probably are no SNPs with large effect sizes. Moreover, a recent study into the heritability of seven skin aging phenotypes estimated the heritability of facial telangiectasia to be low [2]. Thus, for this specific skin aging phenotype, effect sizes of lifestyle related parameters are much greater than effects from genetic variants. Nonetheless, what caught our attention was that multiple highly suggestive hits tagged genes and or expression quantitative trait loci (eQTL) involved in the vascular system, indicating that maybe not all of the suggestive signals are coincidental findings. Replication in other large studies now is required to investigate whether some of these signals could be true. Reproducing GWAS results in other populations remains the gold standard to verify true signals. In practice, however, this is often challenging. Different case definitions can result in heterogeneity of the investigated phenotype, making it more difficult to discover signals with small effect sizes. For example, in **Chapter 2.2** we tried to replicate our findings in an independent German cohort and failed, perhaps due to different phenotype definition or small sample size. This highlights the need for large consortia to be able to reveal genetic backgrounds of traits with low heritability or where multiple genes with small effect sizes are involved. In addition, since GWAS studies are performed within populations of a specific descent, replication in other populations can be difficult. The only SNP significantly associated with telangiectasia in literature was found in Han Chinese women and could not be replicated in our, nor in two other European cohorts, simply because the SNP is not present in Europeans.

WHERE SKIN AGING MEETS HEALTH & DISEASE

The relevance of skin aging research is known to be debated. However, looks substantially impact our psychosocial wellbeing and the skin is an important tool in communication as it displays signs of wealth, sexuality and social status in addition to aging [12]. Moreover, signs of skin aging can be at the border between appearances and health & disease, showing one might not be independent of the other. In **Chapter 3.1** we showed that dry skin affects 60% of the community-dwelling middle-aged to elderly and the prevalence increases with increasing age, indicating that more than half of the individuals is affected by deterioration of the skin barrier. Xerosis may be a (skin aging) feature on its own or it might be a part of a skin condition such as eczema. When suffering from eczema, the probability of having generalized dry skin (GDS) was 7 times as high as when not having eczema. Furthermore, self-perceived health was significantly lower in individuals with a GDS, suggesting there is more to the link between skin health and general health. We found for example that men and women with GDS were more likely to use statins and diuretics, which reflects systemic morbidity, although we cannot exclude xerosis as an adverse effect of these drugs. Overall, GDS is more strongly associated with systemic health parameters than localized dry skin (LDS; mainly xerosis on the extremities). Cream use lowers the risk of LDS but not GDS, acclaiming GDS is a more severe phenotype which is less responsive to external influences. This implies that dry skin on the extremities only (LDS) is a more environmentally influenced or cosmetic condition, whereas with decreasing health the xerosis becomes more severe and widely spread over the body. Furthermore, a GWAS on dry skin only showed significant signals when GDS was used for case-definition, in contrast to analyzing GDS and LDS together, which did not show any signals. This again emphasizes LDS to be only influenced by environmental factors, while GDS is also genetically determined and related to systemic conditions. In **Chapter 3.2** we found several genome-wide significant SNPs associated with GDS, all in the Epidermal Differentiation Complex (EDC) on chromosome 1. The EDC is a large gene-rich region in which many genes are embedded which code for proteins involved in the terminal differentiation and cornification of keratinocytes in the epidermis. In the EDC, linkage disequilibrium (LD) structures are complex, since large genetic regions cluster together, which is often attributed to positive selection. One can imagine that an intact skin barrier, allowing for proper thermoregulation and fluid homeostasis while protecting from pathogens, would be an evolutionary advantage. Nevertheless, LD analysis and eQTL correspondence provided evidence for two separate signals, one tagging the *FLG* (*filaggrin*) gene, the other most closely to the *HRNR (hornerin) gene*. Although plenty evidence for *FLG* gene polymorphisms has been collected in atopic dermatitis (AD) [13] and ichthyosis vulgaris (IV)[14], we revealed the first genetic evidence for genetic susceptibility to dry skin in the general (non-diseased) population. Hence in addition to known non-genetic determinants for GDS (including increasing age, female sex and lower outside temperature) replicated in **Chapter 3.1**, there is

evidence for an additional genetic susceptibility. To verify these signals, replication in another independent cohort is needed.

SKIN AGING AS A POTENTIAL MODEL FOR SYSTEMIC HEALTH

The link between dry skin, aging and skin or systemic diseases, raises the question whether skin aging might reflect systemic aging and vice versa whether healthy habits (known to influence systemic aging) might also influence skin aging. It is known that perceived facial age, or how old one is estimated to look, can be used as a clinical marker and moreover, that it can predict survival. But can systemic aging be read of the face? And could youthful looks be influenced by a healthy diet? In **Chapter 4.1** I showed the association between facial wrinkle percentage and dietary pattern. Interestingly, men and women showed differences in quality as well as in quantity of dietary patterns, which explained the maximum variance in food intake. Women were more likely to consume more fruits and vegetables, whereas men would consume more alcoholic drinks and eat more sweets. Our findings correspond with a study investigating health beliefs in 23 countries where women would tend to eat more healthily and attached greater importance to healthy eating than men [15]. This difference in eating pattern could be the explanation for only finding a link between dietary pattern and wrinkles in women and not in men, where women eat more healthy foods and it's easier to detect such associations. In addition, because of the increased awareness for healthy eating, women could fill in these questionnaires more accurately as compared to men, making the analysis in women more reliable. Differences in recall between men and women when filling in dietary questionnaires may also play a part [16]. Alternatively, differences in skin quality between the sexes could also explain a part of the difference in results since men have thicker skin than women [17] and thick skin is less likely to wrinkle than thinner skin. Women also had 50% more chance to have a GDS than men (**Chapter 3.2**), which also might be explained due to more TEWL in thinner female skin in combination with less sebum production. This skin quality difference might also explain why we only found a link between BMI and telangiectasia in women if female skin is thinner, so more see-through and also more sensitive to increased thinning when greater weight or BMI provides more downforce on facial skin. Female skin, thus, might be more easily influenced by external factors, with dietary pattern being one of them. Reactive Oxygen Species (ROS) from processed foods can damage vital skin components whereas a healthy diet might protect from wrinkling. Flavonoids and vitamins in a healthy diet provide photo protection and stimulate important cellular processes such as collagen production and DNA repair mechanisms [18, 19], which affirms the impact of healthy foods in maintenance of youthful looking skin. Hence, several important cellular processes and mechanisms in our body are activated through consumption of components of certain, mainly healthy, foods. This suggests that our bodies have been well-programmed to process these components. More recently, several nutraceuticals have

emerged claiming a variety of skin care properties [20]. As previously discussed**,** several small supplement studies showed possible beneficial skin effects for e.g. vitamins and carotenoids. The nutraceuticals field is rapidly evolving and new bioactive elements are investigated. Oral collagen supplementation for example, often debated, showed notable skin benefits including increased skin elasticity, hydration and dermal collagen density [21]. Preliminary results in this review showed promising results for oral collagen use in skin wound healing and skin aging. However, more research is needed to establish whether and how this could also be used for skin barrier conditions such as AD and xerosis cutis.

The way we eat today can be considered relatively ultramodern. For the vast majority of human history, humans were hunter-gatherers. A typical hunter-gatherer diet consisted of plenty vegetables, fruits, roots and nuts, supplemented with the occasional fish, meat and eggs. Then, about 10,000 years ago, the largest change in diet in human history took place. Humans transitioned from hunting-gathering to agriculture and animal husbandry. Through these events, mankind was introduced to the dominance of other nutrients such as cereals and dairy, which have never been an important part of their diet before. Over the last two centuries there have been tremendous changes in diet, again. Here, the technical revolution, starting in the late nineteenth century, was the kick start for cheap, quickly available and mass production fast food. Experts in nutrition and evolution hypothesize that these past 10,000 years were too short for our human genome to adapt sufficiently to these new nutrients [22]. With the recent rise in consumption of these 'high calorie' and highly processed foods, there was also a shift in global morbidity. An increase was seen in Western diseases such as diabetes, cardiovascular disease and cancer. Many of these conditions can at least partly be traced back to altered (cell) metabolism and oxidative stress. Some, (e.g. diabetes type II) can even be cured by establishing a normal weight, for example through healthy diet. In addition, obesity is associated with accelerated aging. A large meta-analysis of 87 studies showed shortening of telomeres with increasing BMI, especially in young individuals [23]. Interestingly, not only what we eat is important, but also when we consume it. Intermittent fasting, or abstinence of food during a specific time-frame, has shown to elicit cellular response which improve glucose regulation, increase stress resistance and suppress inflammation, resulting in delayed aging and disease processes [24].

Many of the diseases of our era can be (partly) attributed to poor lifestyle choices, such as high caloric intake, little exercise or smoking. Research examining 137 mummies originating from four distant geographical locations, however, showed that atherosclerosis was common even in preagricultural hunter-gatherers [25] This indicates that although poor lifestyle choices increase the risk of lifestyle-related diseases, not all can be attributed to them. In contrast to that, there are the conditions which occur as a part of the physiological aging of our bodies, or age-related morbidities. In **Chapter 4.2** we showed that youthful looks associated with a lower prevalence of several age-related diseases, suggesting that looks might give away information on healthy aging. Although this should not replace clinical tools and models used to assess

the risk of specific morbidities, it might serve as an additional clinical sign. Finding that looks associate with health does not come as a surprise given that physicians have been using visual cues in physical examination to strengthen their diagnostic considerations for ages. Examples include pallor in anemia or jaundice in liver disease. Looking younger for one's chronological age significantly associated with lower likelihood of cataracts, age-related hearing loss, osteoporosis and COPD, but also with a higher global cognition. Both physical and cognitive health thus seem to be displayed on the face. Even in never-smokers the link between looking younger and less COPD was found, suggesting other mechanisms than smoking are driving this association. Decreased cellularity could play a role where more facial wrinkles, which were driving the association between young looks and age-related diseases, are caused by less fibroblast and osteoporosis by less functional osteoblasts for example. In addition, cell cultures showed that the osteoblast, in fact, is a sophisticated fibroblast [26] hence cell properties are nearly identical. Also, signaling factors such as fibroblast growth factors (FGFs) showed not only to influence skin aging [27] but also bone loss [28].

Nonetheless, decreased cellularity is not the only mechanism which can be hypothesized to be at the basis of the found associations. Depletion of certain DNA repair proteins in both mouse models as well as in cultured human fibroblast showed to increase apoptosis promoted by senescent cells [29]. This might explain premature aging phenotypes and shortened lifespan, which could be driven by impaired DNA repair mechanisms where skin aging could display senescence of internal organs.

We propose that skin aging or youthful looks thus might be used as an easily accessible aging model to understand common aging pathways of several other, less easily accessible organ systems. Alternatively, aged looks might be the mirror of, and part of, the pathway through which systemic morbidity is triggered. Recent studies showed that epidermal dysfunction leads to 'inflammaging', or low grade systemic inflammation, which is linked with the development of aging associated systemic disorders [30]. Correcting this epidermal dysfunction could, hence, provide novel strategies in the prevention of age-related systemic disease.

CONCLUDING REMARKS & FUTURE RECOMMENDATIONS

Different skin aging phenotypes have shown to have different environmental and genetic determinants although some of them cluster together. Skin aging, health and (skin) disease are linked on multiple levels and having youthful looks truly seems to indicate having less age-related morbidity on a multiple organ level. Communalities in aging pathways between skin and other organ systems now need to be more thoroughly established.

Future studies should dive deeper into these links between skin aging and health & morbidity. Longitudinal studies, instead of cross-sectional approaches, might provide additional information on what happens over several years and might help understand aging pathways

throughout the course of time. Large cohorts or consortia are now needed to increase GWAS power and establish more robust evidence for genetic backgrounds. Novel techniques, including artificial intelligence, might help to collect data even more swiftly and accurately, perhaps even making use of photographs on freely accessible social media platforms. Novel studies investigating influences such as that of the microbiome, prebiotics, novel nutraceuticals or air quality on skin aging can provide new insights. Also, different ethnical populations should be included preventing unjustly global extrapolation of research results in selected populations. Interventional studies might be added to the field to examine if and how correcting epidermal dysfunction could improve general health. Altogether, this can help us to establish etiological mechanisms between skin aging and health more accurately, while understanding aging pathways better and expanding the field of skin aging research.

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CHAPTER 5.2

Summary

CHAPTER 1 is a general introduction to this thesis. Skin aging is a complex ensemble of different skin aging phenotypes, including wrinkles, pigmented spots, telangiectasia, sagging and dry skin. Measuring these signs can be done using several compound scores or they can be assessed separately, as they can have different determinants through different etiologies. Hence, epidemiological and genetic determinants can very well differ between different types of skin aging. Telangiectasia and dry skin are less well studied phenotypes and this thesis will focus on the epidemiology and genetic background of these phenotypes using data of the Rotterdam Study (RS). Also, since looking older associates with higher mortality rates, the common ground between skin aging phenotypes and age-related morbidities and other skin aging influencers such as diet is investigated.

CHAPTER 2.1 presents the epidemiology and determinants of facial telangiectasia in the Rotterdam Study. Facial telangiectasia are linear or branched-like blue to red visible vessels, which are typically located on the nose and cheeks. We digitally extracted the percentage telangiectasia which covers the face in 2,842 RS men and women using standardized high resolution facial images and a semi-automated script in MATLAB. Female sex, increasing age, light skin color type, smoking and tendency to develop sunburn were significantly associated with having more facial telangiectasia in a multivariable linear regression model. This study identified potentially new risk factors and successfully replicated determinants previously described to be associated with facial telangiectasia in smaller studies and/or selected populations.

CHAPTER 2.2 presents the exploration of the genetic background of telangiectasia. First, a genome-wide association study (GWAS) was performed to investigate the association between single nucleotide polymorphisms (SNPs) and the percentage telangiectasia covering the face. Secondly, a candidate-gene approach using known pigmentation genes was performed. Telangiectasia were extracted adopting the previously described semi-automated method using high resolution facial photographs. Genotyping was performed using whole blood DNA samples and standardized genotyping protocols. Haplotype Reference Consortium (HRC) 1.1 was used as a reference panel. Our discovery cohort consisted out of 2842 northwestern European men and women. We did not find any genome-wide significant hits (p-value<$5.0x10^{-8}$) associated with telangiectasia. The most significantly associated SNP was rs4417318 (p-value $5.38x10^{-7}$), which mapped to the *SLC16A7* gene on chromosome 12. Other suggestive SNPs tagged genes *ZNF211*, *ZSCAN4*, *ICOS*, and *KCNN3;* SNP eQTLs and phenotype associations tagged links to the vascular system. Nineteen genome-wide suggestive SNPs (p-value <$5.0x10^{-6}$) were tested for replication in a separate cohort of 576 RS men and women and in another replication cohort of 460 elderly women of the German SALIA cohort but none of them were replicated. The pigmentation genes *KIAA0930*, *SLCA45A2* and *MC1R*, were significantly associated with telangiectasia in the candidate gene approach but not independently of skin color. This study suggests that the genetic background of telangiectasia is unclear and remains to be unraveled

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.

CHAPTER 3.1 describes the prevalence and determinants for having a dry skin in the general middle-aged to elderly population. Dry skin was physician scored in 5547 RS men and women as absent, localized dry skin (LDS) or generalized dry skin (GDS) during a full body skin examination (FBSE). Associations with participant characteristics, lifestyle factors, environmental factors, several comorbidities and drug exposure for LDS and GDS were investigated in two separate multivariable logistic regression models. We found that 60 % of our middle-aged to elderly population suffers from dry skin of which one in five had GDS. Increasing age, female sex, light skin color, a lower BMI, lower outside temperature, the presence of eczema and history of chemotherapy were significant determinants for both GDS and LDS. Daily cream use associated with less LDS indicating it is a more easily regulated phenotype than GDS which was not only significantly associated with smoking and the use of statins and diuretics but also with a lower self-perceived health and several dermatological conditions. Having active eczema lesions for example, increased the likelihood of having GDS sevenfold. Our results suggest that dry skin is not only a very common skin condition amongst the middle-aged to elderly but also that dry skin might be an indicator of a person's health status when spread more widely across the body.

CHAPTER 3.2 shows the genetic exploration of dry skin. Here, we scored LDS and GDS as described in chapter 3.1. Genotyping and imputation was performed according to standard protocols using HRC 1.1 as reference panel. First we performed a GWAS labeling both LDS and GDS as cases which resulted in no genome-wide significant results. When excluding the LDS group and labeling only GDS as cases, we found multiple genome-wide significant SNPs on chromosome 1. Rs12123821 (p-value 3.05×10^{-10}) was our top SNP which mapped closest to the *HRNR* gene. Calculation of linkage disequilibrium (LD) between the chromosome 1 top hits showed a long LD block covering the Epidermal Differentiation Complex (EDC), including the *FLG* gene and other EDC genes such as *TCHH*. Conditional analysis demonstrated that rs12123821 was associated independently of *FLG* gene SNPs and was not driven by co-occurrence of eczema. Genome-wide highly suggestive hits (p-value$<5.0 \times 10^{-7}$) included hits on chromosome 2, 16 and 18. SNP rs75687828 (p-value 3.70×10^{-7}) on chromosome 16 did not associate independently of *MC1R* gene SNPs. In conclusion, we identified multiple SNP's on chromosome 1 that are genome-wide significantly associated with a generalized dry skin phenotype in the general population. This study supports a role for a polygenetic predisposition to the dry skin spectrum where EDC genes play a role in its genetic susceptibility.

CHAPTER 4.1 aims at investigating the association between diet and facial wrinkles in the middle-aged to elderly population. Facial wrinkles were semi-automatically extracted using

high resolution images and previously described methods. Diet was assessed using a validated semi-quantitative Food Frequency Questionnaire (FFQ). The items of the FFQ were bundled in 34 food groups by a nutritionist, based on their nutritional composition. Principal Component Analysis (PCA) of these food groups revealed respectively four and three food patterns in women and in men. In addition, adherence to the Dutch Healthy Diet Index (DHDI) was calculated, which represents how well one abides by the Dutch governmental recommendations for a healthy diet. Associations between diet and facial wrinkles were assessed in a total of 2,753 men and women using multivariable linear regression. Better adherence to the DHDI was significantly associated with less wrinkles in women but not in men. In women, a red meat and snack dominant PCA pattern associated with more wrinkles whereas a fruit dominant PCA pattern associated with fewer wrinkles. Our study suggests that dietary habits associate with facial wrinkling in women but not in men. Global disease prevention strategies might benefit from emphasizing that a healthy diet is also linked to less facial wrinkles.

CHAPTER 4.2 dives into the link between aging on the outside versus aging on the inside. Looking older associates with dying younger. To unravel which aging mechanisms might be involved and how aging of different organ systems correlates with an aged appearance, we looked into the association between how old an individual looks, given their chronological age and age-related morbidities of the elderly. Perceived age was scored in 2679 RS men and women by a panel of assessors which were blinded to chronological age. The delta between the mean perceived age and chronological age was calculated (ΔPA), where a higher (positive) ΔPA means the subjects looks younger for his age and a lower (negative) ΔPA that the person looks older. ΔPA was tested as a continuous variable for association with presence of the most prevalent and characteristic age-related cardiovascular, pulmonary, ophthalmological, neurocognitive, renal, skeletal and auditory morbidities in separate regression analyses, while adjusting for known confounders. We observed a higher ΔPA (i.e., looking younger for age) associated with less osteoporosis, less COPD, less age related hearing loss, less cataracts, while with better global cognitive functioning. Our results show that looking 'young for your age' is truly a clue for having less systemic morbidity and better cognitive function. More research is needed to confirm which aging mechanisms are involved exactly. Overall, our study underlines perceived facial age as a biomarker for healthy aging in northwestern Europeans.

CHAPTER 5.1 is the general discussion to this thesis. Here the most important conclusions of our research are presented and implications for future research are given. Epidemiological determinants for different skin aging phenotypes are compared and differences and commonalities between them discussed. This knowledge could be used to understand different mechanisms of skin aging but also to develop customized or tailored prevention measures for the individual. The skin aging field is expanded towards skin physiology and disease when investigating determinant of xerosis cutis. Genetics and heritability differ between skin aging

phenotypes and genetic predisposition seems more important for example for dry skin than for telangiectasia. Skin aging and health are linked on multiple levels. Aging of the skin might even be influenced or slowed down via healthy diet. When widely spread across the body, dry skin might be an indicator of a person's health and youthful looks show to be associated with less age-related morbidity. These findings underline the skin as a communicating organ which might be used to study systemic aging, if communalities in aging pathways are further identified. Future skin aging research might include longitudinal studies to better understand aging patterns over time and large consortia can help identify genetic backgrounds for phenotypes with small genetic influences. Research into the skin microbiome or air quality might be the next step in skin aging research and novel techniques such as artificial intelligence may help to collect data swiftly and accurately.

CHAPTER 5.3

Nederlandse samenvatting

Hoofdstuk 1 is de algemene introductie van dit proefschrift. Huidveroudering is een complex samenspel van verschillende huidverouderingsfenotypes, waaronder rimpels, pigmentvlekken, teleangiëctastieën, verslapping en droge huid. Het meten van deze tekenen van huidveroudering kan worden verricht met behulp van een verscheidenheid aan samengestelde scores of de fenotypes kunnen apart onderzocht worden aangezien ze verschillende determinanten kunnen hebben op grond van andere etiologieën. Dat betekent dat epidemiologische en genetische determinanten wel eens zouden kunnen verschillen tussen de verschillende fenotypes van huidveroudering. Teleangiëctastieën en droge huid zijn minder goed bestudeerde fenotypes en dit proefschrift zal zich richten op de epidemiologische en genetische achtergrond van deze fenotypes, waarbij we gebruik maken van data uit de Rotterdam Studie (RS). Daarnaast weten we dat 'er oud uitzien voor de leeftijd' geassocieerd is met een hogere mortaliteit. Dit was reden om ons verder te verdiepen in de raakvlakken tussen huidveroudering en ouderdomsziektes en om te kijken naar andere mogelijke invloeden op huidveroudering zoals dieet.

Hoofdstuk 2.1 richt zich op de epidemiologie en de determinanten van teleangiëctastieën in het gelaat. Teleangiëctastieën in het gelaat zijn lineaire of vertakkende blauw tot rode zichtbare bloedvaatjes, welke zich meestal op de neus en wangen bevinden. Met behulp van een digitale methode en een semiautomatisch script in MATLAB hebben we uit gestandaardiseerde foto's van hoge resolutie van 2,842 RS mannen en vrouwen het percentage van het gezicht wat bedekt is met teleangiëctastieën kunnen extraheren. Determinanten die geassocieerd waren met het hebben van meer teleangiëctastieën in het gelaat in een multivariabel lineair regressie model waren vrouwelijk geslacht, toenemende leeftijd, licht huidskleur type, roken en 'snel verbranden in de zon'. Deze studie heeft met succes determinanten voor teleangiëctastieën gerepliceerd die eerder waren beschreven in beperkte populaties en tevens mogelijk nieuwe risicofactoren ontdekt.

Hoofdstuk 2.2 geeft de verkenning van de genetische achtergrond van teleangiëctastieën weer. Allereerst hebben we een genoomwijde associatie studie (GWAS, "genome-wide association study") verricht, om het verband tussen enkel-nucleotide polymorfismen (SNPs, "single-nucleotide polymorphisms") en het percentage teleangiëctastieën in het gelaat te onderzoeken. Ten tweede werd een kandidaatgen benadering gekozen waarbij gebruik werd gemaakt van bekende pigmentatie genen. Teleangiëctastieën werden op de eerder genoemde gestandaardiseerde wijze geëxtraheerd. Het genotyperen werd verricht gebruik makende van volbloed DNA monsters en gestandaardiseerde protocollen voor genotypering. Haplotype Reference Consortium (HRC) 1.1. werd gebruikt als referentiepanel. Ons ontdekkingscohort bestond uit 2,842 Noordwest Europese mannen en vrouwen. We hebben geen genoomwijd significante (p-waarde$<5.0\times10^{-8}$) signalen gevonden geassocieerd met teleangiëctastieën. De meest significant geassocieerde SNP was rs4417318 (p-waarde 5.38×10^{-7}) welke werd gelinkt aan het *SLC16A7* gen op chromosoom 12. Andere suggestieve SNPs (p-waarde $<5.0\times10^{-6}$) werden gelinkt aan de

genen *ZNF211, ZSCAN4*, *ICOS* en *KCNN3*. SNP-gerelateerde expressie-kwantitatieve kenmerk-loci (eQTL, "expression quantitative trait loci") en fenotype associaties lieten verbanden zien met het vasculaire systeem. Negentien genoomwijd suggestieve SNPs (p-waarde $<5.0x10^{-6}$) werden getest op replicatie in een afzonderlijk cohort van 576 RS mannen en vrouwen en in een ander replicatiecohort van 460 oudere vrouwen van het Duitse SALIA-cohort, echter zonder succes. Pigmentatie genen *KIAA0930*, *SLCA45A2* en *MC1R* waren significant geassocieerd met teleangiëctastieën in de kandidaatgen benadering, echter niet onafhankelijk van huidskleur. Deze studie suggereert dat de genetische achtergrond van teleangiëctastieën onduidelijk is en nog moet worden ontrafeld, hoewel suggestieve signalen aangeven dat genen die betrokken zijn bij het vasculaire systeem mogelijk een rol spelen. De significant geassocieerde pigmentatie genen benadrukken het verband tussen huidskleur en teleangiëctastieën.

In **hoofdstuk 3.1** beschrijven we de prevalentie van droge huid en de determinanten ervoor in de algemene populatie van middelbare tot oudere leeftijd. Droge huid is door een getrainde arts geobjectiveerd bij 5,547 RS mannen en vrouwen en genoteerd als geen droge huid, gelokaliseerd droge huid (LDS, "localized dry skin") of generaliseerd droge huid (GDS, "generalized dry skin") gedurende een onderzoek van de gehele huid. Associaties met deelnemer karakteristieken, leefstijl factoren, omgevingsfactoren, diverse comorbiditeiten en de blootstelling aan medicijnen werd onderzocht in verband met LDS en GDS in twee afzonderlijke multivariabele logistische regressie modellen. We vonden dat 60% van onze populatie van middelbare tot oudere leeftijd last had van een bepaalde mate van droge huid, van wie één op vijf GDS had. Significante determinanten voor zowel LDS als GDS waren toenemende leeftijd, vrouwelijk geslacht, lichte huidskleur, lagere 'body mass index' (BMI), lagere buiten temperatuur, aanwezigheid van eczeem en chemotherapie in het verleden. Het dagelijks aanbrengen van crème was geassocieerd met minder LDS, wat suggereert dat het een makkelijker te reguleren fenotype is dan GDS wat op zijn beurt niet alleen geassocieerd was met roken en het gebruik van statines en diuretica maar ook met een lagere zelf ingeschatte gezondheid en diverse dermatologische aandoeningen. Het hebben van actieve eczemateuze laesies bijvoorbeeld, verhoogde de kans op het hebben van GDS maar liefst zeven keer. Onze resultaten suggereren dat droge huid niet alleen een zeer vaak voorkomende aandoening is bij mensen van middelbare tot oudere leeftijd, maar ook dat droge huid mogelijk een indicator kan zijn voor iemands gezondheid indien meer wijd verspreid over het lichaam.

In **hoofdstuk 3.2** beschrijven we de genetische exploratie van droge huid. LDS en GDS werden bepaald zoals beschreven in **hoofdstuk 3.1**. Genotypering en imputatie werden verricht volgens standaard protocollen, waarbij HRC 1.1 werd gebruikt als referentiepanel. Allereerst hebben we een GWAS verricht waarbij zowel LDS als GDS als cases werden aangeduid, wat resulteerde in geen genoomwijd significante resultaten. Ten tweede werd de LDS groep geëxcludeerd waarbij alleen de GDS groep werd aangeduid als cases en geen droge huid als controles. Hierbij vonden

we multipele significante SNPs op chromosoom 1. Rs12123821 (p-waarde $3.05x10^{-10}$) was onze meest significante SNP, welke gelokaliseerd werd naast het HRNR gen. Bij het onderzoeken van de onderlinge verwantschap tussen de SNPs op chromosoom 1, werd er een groot verwant genetisch gebied geïdentificeerd die het epidermale differentiatie complex (EDC) omvatte, inclusief de genen *FLG* en andere EDC genen zoals *TCHH*. Conditionele analyses toonden dat rs12123821 onafhankelijk van *FLG* gen SNPs geassocieerd was met GDS en dat de SNP niet gedreven werd door het gelijktijdig voorkomen van eczeem. Genoomwijd suggestieve signalen (p-waarde<$5.0x10^{-7}$) werden gevonden op chromosomen 2, 16 en 18. SNP rs75687828 (p-waarde $3.70x10^{-7}$) op chromosoom 16 was niet onafhankelijk van *MC1R* gen SNPs geassocieerd met droge huid. Concluderend, we hebben multipele SNPs geïdentificeerd op chromosoom 1, die genoomwijd significant geassocieerd zijn met een gegeneraliseerd droge huid fenotype in de algemene bevolking. Deze studie ondersteunt een rol voor een polygenetische predispositie in het droge huid spectrum waarbij EDC genen een rol spelen.

In **Hoofdstuk 4.1** hebben we gepoogd om het verband tussen voedingspatroon en rimpels in het gelaat te onderzoeken in de algemene bevolking van middelbare tot oudere leeftijd. Rimpels in het gelaat werden semiautomatisch gekwantificeerd gebruik makende van foto's van hoge resolutie en eerdergenoemde methodes. Voedingspatroon werd beoordeeld met behulp van een gevalideerde semi-kwantitatieve voedsel frequentie vragenlijst (FFQ, "food frequency questionnaire"). De items van de FFQ werden vervolgens in 34 groepen gebundeld door een diëtist, gebaseerd op de voedingseigenschappen. Principale componenten analyse (PCA) van deze 34 voedselgroepen leverde respectievelijk 4 voedselpatronen op in vrouwen en 3 in mannen. Daarnaast werd de score op de DHDI ("Dutch Healthy Diet Index") berekend, wat weergeeft hoe goed iemand zich houdt aan de Nederlandse overheidsrichtlijnen voor gezonde voeding. De associaties tussen voedingspatroon en rimpels in het gelaat werden onderzocht in een groep van 2,753 RS mannen en vrouwen met behulp van lineaire regressie. Een hogere score op de DHDI en zich beter houden aan de Nederlandse overheidsrichtlijnen voor een gezonde voeding was geassocieerd met minder gelaatsrimpels bij vrouwen, maar niet bij mannen. Bij vrouwen was tevens een PCA patroon bestaande uit veel rood vlees en snacks geassocieerd met meer rimpels, terwijl een fruit gebaseerd PCA patroon verband hield met significant minder rimpels. Onze studie suggereert dat voedingspatroon geassocieerd is met rimpels in het gelaat bij vrouwen maar niet bij mannen. Wereldwijde ziekte preventie strategieën zouden kunnen profiteren van het benadrukken dat een gezond dieet ook verband houdt met minder rimpels in het gelaat.

In **hoofdstuk 4.2** duiken we dieper in het verband tussen veroudering aan de buitenkant en veroudering van de binnenkant. Er ouder uitzien voor de leeftijd is geassocieerd met eerder overlijden. Om te ontrafelen welke verouderingsmechanismen hier aan ten grondslag liggen en hoe het verouderen van verschillende orgaansystemen correleert met een verouderd uiterlijk,

hebben we gekeken naar het verband tussen 'hoe oud iemand er uitziet voor de leeftijd' en diverse ouderdomsgerelateerde aandoeningen. Geschatte leeftijd (PA, "perceived age") werd bepaald in 2,679 RS mannen en vrouwen door een panel van beoordelaars die geblindeerd waren voor chronologische leeftijd. De delta (Δ) tussen de gemiddelde PA en chronologische leeftijd (ΔPA) werd berekend waarbij een hogere (positieve) ΔPA betekende dat de persoon er jonger uitzag voor de leeftijd en een lagere (negatieve) ΔPA dat diegene er ouder uitzag. ΔPA werd getest als een continue variabele geassocieerd met de aanwezigheid van vaak voorkomende en typisch ouderdomsgerelateerde aandoeningen van het cardiovasculaire, pulmonale, oftalmologische, neurocognitieve, renale, skeletale en auditoire orgaansysteem in afzonderlijke regressie analyses waarbij gecorrigeerd werd voor de meest belangrijke confounders. We vonden dat een hogere ΔPA (er jonger uitzien voor de leeftijd) geassocieerd was met minder osteoporose, minder COPD, minder leeftijdsgerelateerd gehoorverlies, minder cataract en met betere globaal cognitief functioneren. Onze resultaten laten zien dat 'er jong uitzien voor de leeftijd' werkelijk een teken is voor minder systemische morbiditeit en beter cognitief functioneren. Meer onderzoek is nodig om te bevestigen welke verouderingsmechanismen exact zijn betrokken. Onze studie onderstreept PA als een biomarker voor gezond ouder worden in de noordwest Europese populatie.

Hoofdstuk 5.1 is de generale discussie van deze thesis. Hier worden de meest belangrijke conclusies van ons onderzoek uiteengezet en aanbevelingen voor toekomstig onderzoek worden gegeven. Epidemiologische determinanten voor de verschillende huidverouderingsfenotypes worden vergeleken en onderlinge verschillen en overeenkomsten besproken. Deze informatie kan gebruikt worden om de verschillende mechanismen van huidveroudering te doorgronden en tevens om op maat gemaakte anti- huidverouderingsmaatregelen te ontwikkelen voor het individu. Het onderzoeksgebied van huidveroudering is uitgebreid in de richting van huidfysiologie en ziekte met het onderzoek van determinanten voor de droge huid. Genetica en erfelijkheid verschillen tussen huidverouderingsfenotypes en genetische predispositie lijkt bijvoorbeeld een grotere rol te spelen in de ontwikkeling van een droge huid dan voor teleangiëctastieën. Huidveroudering en gezondheid zijn verwant op multipele niveaus. Huidveroudering kan wellicht zelfs beïnvloed worden of geremd door een gezond voedingspatroon. Indien wijd verspreid over het lichaam, kan droge huid een indicator zijn van iemands gezondheidsstatus en 'er jong uitzien voor de leeftijd' blijkt geassocieerd te zijn met minder ouderdomsgerelateerde aandoeningen. Deze bevindingen benadrukken de rol van de huid als communicerend orgaan hetgeen gebruikt zou kunnen worden om systemische veroudering te bestuderen, als overeenkomsten in mechanismen van veroudering verder geïdentificeerd worden. Toekomstig huidverouderingsonderzoek zou meer longitudinale studies kunnen toevoegen om verouderingspatronen door de tijd heen beter te kunnen begrijpen en grotere consortia zouden kunnen bijdragen in het ontrafelen van de genetische basis van fenotypes met kleine genetische effecten. Onderzoek van het microbioom van de huid of van luchtkwaliteit zou de volgende stap kunnen zijn in

huidverouderingsonderzoek en nieuwe technieken zoals kunstmatige intelligentie (AI, “artificial intelligence”) zouden kunnen helpen om data snel en accuraat te verzamelen.

CHAPTER 5.4

List of co-authors

List of publications

Abbreviations

PhD portfolio

Curriculum Vitae

Dankwoord

LIST OF COAUTHORS

A. Goedegebure, PhD, Department of Otorhinolaryngology, Head and Neck Surgery, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

A.E. Mayes, PhD, Unilever Research and Development, Colworth Science Park, Sharnbrook, United Kingdom

A.E.G. Haarman, MD, Departments of Epidemiology and Ophthalmology Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

B. Arshi, researcher, Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands

C.C.W. Klaver, Ophthalmologist and epidemiologist, Professor of Epidemiology and the Genetics of Eye Diseases, Departments of Epidemiology and Ophthalmology Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

C. Wigmann, PhD, IUF, Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

D.A. Gunn, PhD, Unilever Research and Development, Colworth Science Park, Sharnbrook, United Kingdom

D.J. Hijnen, Dermatologist, Associate professor, Department of Dermatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

E.F. Thee, MD, Departments of Epidemiology and Ophthalmology Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

E.J. Vinke, PhD, Departments of Epidemiology and Radiology and Nuclear Medicine, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

E.W. de Roos, MD, Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands

F. Rivadeneira, MD, PhD, Department of Internal Medicine, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

G.G.O. Brusselle, Pulmonologist, Professor of Medicine, Ghent University Hospital, Ghent, Belgium

J.B.J. van Meurs, PhD, associate professor, Department of Internal Medicine, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

J.C. Kiefte- de Jong, Dietitian, Professor of Population Health, Leiden University Medical Center, Leiden, the Netherlands

J.D. Schoufour, PhD, Departments of Internal Medicine and Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

J.E. Vergroesen, MD, Department of Ophthalmology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

K. Trajanoska, PhD, Departments of Epidemiology and Internal Medicine, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands

L.C. Jacobs, Dermatologist, PhD, Department of Dermatology, Amphia Ziekenhuis, Breda, the Netherlands

L. M. Pardo Cortes, MD, PhD, Department of Dermatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

M.A. Hamer, MD, PhD, Department of Dermatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

M.A. Ikram, Radiologist, Professor of Epidemiology and Radiology, Head of Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

M. Kavousi, Associate Professor, Department of Epidemiology, Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands

M. Kayser, Professor of Forensic Molecular Biology, Head of department of Genetic Identification, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands,

M.W. Vernooij, Radiologist, Professor of Population Imaging, Department of Radiology and Nuclear Medicine, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

P.H. Croll, PhD, Epidemiologist, Departments of Epidemiology, Radiology and Nuclear Medicine and Otorhinolaryngology, Head and Neck Surgery, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

T.E.C. Nijsten, Dermatologist, Professor of Dermatology, Head of department of Dermatology, Erasmus MC University Medical Center Rotterdam, Rotterdam, the Netherlands

T. Schikowski, PhD, Environmental Epidemiologist, Head of research group, IUF, Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

LIST OF PUBLICATIONS

Mekić S, Jacobs LC, Hamer MA, Ikram MA, Schoufour JD, Gunn DA, Kiefte-de Jong JC, Nijsten T. A Healthy Diet in Women Is Associated With Less Facial Wrinkles in a Large Dutch Population-Based Cohort. *J Am Acad Dermatol 2019 May; 80(5):1358-1363.*

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Luijnenburg SE, **Mekić S**, van den Berg J, van der Geest RJ, Moelker A, Roos-Hesselink JW, Bogers AJ, de Rijke YB, Strengers JL, Mulder BJ, Vliegen HW, Helbing WA. Ventricular Response to Dobutamine Stress Relates to the Change in Peak Oxygen Uptake During the 5-year Follow-Up in Young Patients With Repaired Tetralogy of Fallot. *Eur Heart J Cardiovasc Imaging. 2014 Feb; 15(2):189-94.*

Veldhorst MA, Noppe G, Jongejan MH, Kok CB, **Mekić S**, Koper JW, van Rossum EF, van den Akker EL. Increased Scalp Hair Cortisol Concentrations in Obese Children. *J Clin Endocrinol Metab. 2014 Jan; 99(1)285-90*

ABBREVIATIONS

AD	Atopic dermatitis
AI	Artificial intelligence
ARHL	Age-related hearing loss
BMI	Body mass index
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
eQTL	Expression quantitative trait locus
FBSE	Full body skin examination
FGFs	Fibroblast growth factors
GDS	Generalized dry skin
GWAS	Genome-wide association study
IV	Ichthyosis vulgaris
IQR	Interquartile range
LD	Linkage disequilibrium
LDS	Localized dry skin
LLS	Leiden Longevity Study
MMPs	Matrix metalloproteinases
OR	Odds ratio
PA	Perceived age
PCA	Principal component analysis
ROS	Reactive oxygen species
RS	Rotterdam Study
UV	Ultraviolet
SALIA	Study on the Influence of Air Pollution on Lung, Inflammation and Aging
SD	Standard deviation
SE	Standard error
SNP	Single-nucleotide polymorphism
TEWL	Transepidermal water loss
95%CI	95% confidence interval
ΔPA	Delta perceived age
%Δ	Percentage change

PHD PORTFOLIO

Name PhD Student:	Selma Mekic
PhD period:	2017-2020
Promotor:	Prof. dr. T.E.C. Nijsten
Co-promotor:	dr. L.C. Jacobs
	dr. L.M. Pardo-Cortes

Activity	Year	Workload
Courses		
NIHES: Principles of Epidemiologic Data-analysis	2017	0.7 ECTS
NIHES: Biostatistical methods I: Basic Principles A	2017	2.0 ECTS
NIHES: Regression analysis	2017	1.4 ECTS
NIHES: Genome-wide Association Studies	2017	0.7 ECTS
BROK good clinical practice	2018	1.5 ECTS
Molmed: Presenting skills for junior researchers	2018	1.0 ECTS
NIHES: GWAS Blitz course	2019	2.0 ECTS
Conferences attended		
1st European Dermato-epidemiology Network(EDEN), Madrid, Spain	2017	1.0 ECTS
5th PhD weekend Erasmus MC Dermatology, den Bosch, the Netherlands	2017	1.0 ECTS
2nd European Dermato-epidemiology Network (EDEN), Berlin, Germany	2018	1.0 ECTS
19th Annual meeting Nederlandse Vereniging voor Experimentele Dermatologie (NVED), Lunteren, the Netherlands	2018	1.0 ECTS
BBSRC Student Workhop Conference, Unilever Research and Development, Colworth Science Park, Sharnbrook, United Kingdom	2018	1.0 ECTS
6th PhD weekend Erasmus MC Dermatology, Breda, the Netherlands	2018	1.0 ECTS
Najaarssymposium Nederlandse Vereniging voor Cosmetische Dermatologie (NVCD), Bussum, the Netherlands	2018	1.0 ECTS
10th Porto Skin Challenges 2019, Porto, Portugal	2019	1.0 ECTS
28th European Academy of Dermatolgy and Vernereology (EADV) Congress, Madrid, Spain	2019	1.0 ECTS
7th PhD weekend Erasmus MC Dermatology, Scheveningen, the Netherlands	2019	1.0 ECTS
Oral presentations		
Introduction to thesis, Skintermezzo Erasmus MC, Rotterdam	2017	1.0 ECTS
Thesis overview, BBSRC student workshop, Unilever R&D, Colworth Science Park, Sharnbrook	2018	1.0 ECTS
Skin aging, Epidemiology, determinants and genetics, 2020 meeting, department of Epidemiology, Erasmus MC, Rotterdam	2019	1.0 ECTS
Epidemiology and determinants of facial telangiectasia, a cross-sectional study, Porto skin challenges 2019, Porto	2019	1.0 ECTS

'Littekenbehandeling op de poli' -Dutch Aesthetic Laser Association (DALA), Erasmus MC, Rotterdam	2019	1.0 ECTS
Poster presentations		
A healthy diet in women is associated with less facial wrinkles, in a large Dutch population-based cohort, presented at EDEN Berlin, NVED Lunteren and Unilever BBSRC Student Workhop, Colworth Science Park	2018	3.0 ECTS
Teaching		
Research onderwijs: 'Ordinal regression'	2018	1.0 ECTS
ICK onderwijs: 'Snijden, hechten en biopteren'	2018-2019	1.0 ECTS
Research onderwijs: 'Genetics in skin aging'	2019	1.0 ECTS
Other		
PhD weekend organizing committee	2018	1.0 ECTS
Teambuilding dept. of dermatology 'Vrijmibo'	2019-2020	1.0 ECTS
Setting up scar outpatient clinic, dept of dermatology Erasmus MC	2019-2020	2.0 ECTS

CURRICULUM VITAE

Selma Mekić werd geboren op 9 februari 1990 te Zvornik in voormalig Joegoslavië, het hedendaagse Bosnië-Herzegovina. Na het uitbreken van de oorlog, vluchtte zij samen met haar ouders en broer en kwam terecht in het zuiden van Nederland, waar zij tot haar 18e is verbleven. Na het afronden van het atheneum aan de Regionale Scholengemeenschap 't Rijks in Bergen op Zoom, vertrok zij in 2008 naar Rotterdam voor de studie geneeskunde aan de Erasmus Universiteit. Gedurende de studie geneeskunde waren haar aandachtsgebieden uiteenlopend. De interesse in gezondheid en beweging leidde tot een minor in Sportgeneeskunde en Revalidatie in 2011 aan de Erasmus Universiteit. Het masteronderzoek verrichtte zij in 2012 in het Sophia kinderziekenhuis te Rotterdam waarbij het stresshormoon in haren werd gemeten in kinderen met overgewicht. Gedurende haar studie was Selma werkzaam in het studententeam van de radiologie waar de interesse in minimaal invasieve behandelingen ontstond. In de weekenden werkte ze daarnaast bij Parfumerie Douglas te Bergen op Zoom waar haar belangstelling voor huidverzorging en esthetiek werd gewekt. Kennismaking met het vak dermatologie in de coschappen zorgde ervoor dat de puzzelstukjes van interesse op hun plaats vielen. De studie geneeskunde werd in 2015 afgerond met eind 2014 aansluiting bij de minor Global Health in Kenia met stages in chirurgie en tropendermatologie. Na het behalen van het artsenexamen in 2015 werkte zij anderhalf jaar als ANIOS interne geneeskunde in het Albert Schweitzer Ziekenhuis te Dordrecht en Zwijndrecht. Begin 2017 startte zij met haar promotie traject op de afdeling dermatologie van het Erasmus MC. Hierbij mocht zij de lijn van het huidverouderingsonderzoek op de afdeling dermatologie van het Erasmus MC voortzetten, onder begeleiding van promotor Prof. dr. T. Nijsten en copromotoren dr. L. Jacobs en dr. L. Pardo. Naast het schrijven van huidige thesis heeft zij samen met dr. P. Velthuis in het Erasmus MC een specialistisch spreekuur opgezet voor de behandeling van littekens. Per 1 mei 2020 is zij gestart met de opleiding tot dermatoloog in het Universitair Medisch Centrum te Utrecht. Selma woont samen met Bram Willemse in hun huis in Rotterdam West.

DANKWOORD

We dit it! Het is klaar, af, WOEEHOEEEEEEEEEE. Alleen had ik dit natuurlijk nooit gekund, daarom wil ik graag nog een paar mensen bedanken.

Prof. Nijsten, beste Tamar. Ik kan me nog haarscherp herinneren dat ik net klaar was met de studie geneeskunde en dat ik een keer mocht komen kennismaken. Heel spannend vond ik dat. Je legde me alle verschillende takken van het onderzoek binnen de dermatologie in het EMC, beknopt maar toch illustratief uit. Rondkijkend in het indrukwekkende kantoor in het GK-gebouw, waar je niet al te lang daarvoor je intrede had genomen, voelde ik intuïtief meteen al een voorkeur voor de onderzoekstak van skin aging. Toen ik je vertelde dat ik mijn eerste ANIOS baan bij de interne geneeskunde zojuist had geaccepteerd, moedigde je dat juist aan. Enigszins vertwijfeld en wat onzeker, verliet in jouw kantoor. We hielden af en toe contact en ruim een jaar later was er toch een plekje vrij gekomen in juist de skin aging groep. Ik kon mijn geluk niet op! De jaren vol wetenschap zijn voorbij gevlogen. In het begin had ik moeite om mijn rust te pakken en echt voor het onderzoek te gaan zitten. Mijn geluk was dat ik kon opstappen op een goed onderhouden, rijdende trein, dus het onderzoek kon vervolgd worden als een goed geoliede machine. Dankjewel Tamar, dat je me deze kans hebt gegeven. Ik heb denk ik meer van je geleerd dan dat jij je beseft. Een beetje kritisch literatuur lezen ja, dat is een open deur. Maar wat minder voor de hand liggend; een stukje weerbaarheid en kwetsbaar durven op te stellen, met andere woorden, persoonlijke groei. Als ik 3 termen moest noemen die jouw begeleiding door de jaren heen typeren: dakpannenstructuur, helikopterview en hap-snap. Kortom, je hebt me geholpen het wetenschappelijke kaf van het koren te leren scheiden en dichter bij mezelf te staan. Wie weet, tot ziens in de toekomst.

Beste Luba, dankjewel voor je begeleiding als copromotor. Je hebt veel geduld met me moeten opbrengen toen ik met de genetische analyses meerdere keren per dag naar je toe kwam. Met je engelengeduld (en veel Spaanse termen die ik zo snel niet kon volgen) wist je het elke keer op te lossen. Dit vond je leuk om te doen en dat was te zien. We hebben het elkaar niet altijd even makkelijk gemaakt, waarschijnlijk door een Colombiaans vs Balkan temperament ;), maar we hebben ook leuke tijden gekend zoals dat je mee ging borrelen in de Kraft bar en het congres in Porto, waar we elke avond uitgebreid met zijn tweeën uit eten gingen en urenlang konden kletsen. Ik heb veel van je kunnen leren op het gebied van genetica en programmeren.

Lieve Leonie, of moet ik zeggen, de grondlegster van het skin aging onderzoek. Wat een feest dat jij van het begin van mijn onderzoek mijn copromotor mocht zijn! Vanaf dag 1 keek ik onwijs tegen je op en nu nog steeds. Zo jong en slim en al gepromoveerd. Ik mocht altijd bij je aankloppen. De periodieke begeleidingen gingen altijd gepaard met een goed bakje koffie en wat lekkers. Naast al dat gezelligs ben je ook een doortastende en zo nodig kritische begeleider,

wel altijd met een grote glimlach :). Ik heb je altijd gemist vanaf toen je begin 2019 klaar was met je opleiding en naar het Amphia vertrok om daar als dermatoloog te werken. We bleven elkaar echter regelmatig zien op jouw parttime dag. Koffietentje hier en lunchroom daar, je was nooit te beroerd om tijd voor me vrij te maken. Manuscripten las je 's avonds na je drukke poli's of in het weekend door, waarbij je deze altijd naar een hoger niveau wist te tillen. Ik ben je erg dankbaar voor je warme begeleiding en hoop je nog vaak te zien. Liefs, ook aan Kevin en Jorn.

Dokter Velthuis, lieve Peter, jij mag natuurlijk niet ontbreken in dit lijstje. Na een tijdje onderzoek te hebben gedaan miste ik toch wel erg het patiënten contact. Tamar begreep dit gelukkig goed en stelde ons al snel aan elkaar voor. Jij, het begrip op cosmetisch dermatologisch gebied in Nederland, was pas in het Erasmus MC komen werken en had inmiddels Erasmus MC aesthetics opgericht. Altijd vol met nieuwe ideeën, plannen, en start-ups, ben je denk ik de leukste supervisor die een A(N)IOS/arts-onderzoeker zich kan wensen. Samen hebben we het littekenspreekuur opgericht waarbij ik zo veel verschillende behandeltechnieken van je heb mogen leren, het is absurd. Altijd had je tijd, maar bovenal ook zin en er plezier in om me iets bij te brengen. Tussen de poli's door zat je aan je wiskunde huiswerk, want naast je werk deed je er ongeveer nog 27385239 dingen bij, gewoon omdat je dat leuk vindt om te doen. Met recht, ben je een bezige bij te noemen. En als ik je nodig had, was je er altijd. Ik vind het jammer dat ik maar zo kort van je heb mogen leren. Dankjewel lieve Peter. P.S. ik denk nog regelmatig aan je als ik de Alzheimer sokken aantrek.

Dear Dave, I loved your guidance. Thank you for immensely improving every single one of our papers. With your ever clever, analytical and above all, very polite suggestions for improvement, it was (almost) a joy to do revisions. I am happy I got to visit you and meet the Unilever team, I had a great time in Sharnbrook!

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Mijn paranimfen; jongens; met jullie is het altijd Time To Shine!

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wilde westen, wat maar een paar minuten fietsen is. Milano happened. Er braken gouden tijden aan, met frequent overleg op de late vrijdagmiddag, op de trappen, in de zon. De jaren vlogen voorbij en ondanks dat je weer naar Dordrecht vertrok, kwamen we juist dichter bij elkaar. Ik wil je bedanken dat je er altijd voor me bent, zonder oordeel en met je ontzettend scherpe analytische blik, kan ik het met jou altijd over alles hebben. Plus het is altijd gezellig, zelfs rond het middaguur in een skate parkje in de zon. Ben super blij dat jij achter mij komt te staan en dat ik een paar weken daarvoor natuurlijk ook achter jou mag staan!

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Andel en Esra, we go way back. Way back. Betere cheerleaders heb en ken ik niet. Thanks for always having my back flappies. Met jullie is altijd alles exxtraaa.

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