Lifestyle and physiological factors associated with facial wrinkling in men and women

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ABSTRACT

Facial wrinkling is one of the most notable signs of skin aging. Men and women show different wrinkling patterns yet the lifestyle and physiological factors underlying these sex-specific patterns are relatively unknown. Here, we investigated sex-specific determinants for facial wrinkles. Wrinkle area was quantified digitally using facial photographs of 3831 northwestern Europeans (51-98 years, 58% female). Effect estimates from multivariable linear regressions are presented as the percentage difference in the mean value of wrinkle area per unit increase of a determinant (%Δ). Wrinkle area was higher in men (median 4.5%, interquartile range (IQR) 2.9-6.3) than in women (3.6%, IQR 2.2-5.6). Age was the strongest determinant, and current smoking (men: 15.5%Δ; women: 30.9%Δ) and lower body mass index (men: 1.7%Δ; women: 1.8%Δ) were also statistically significantly associated with increased wrinkling. Pale skin color showed a protective effect (men: -21.0%Δ; women: -28.5%Δ) and, in men, sunburn tendency was associated with less wrinkling. In women, low educational levels and alcohol use were associated with more wrinkling, whereas female pattern hair loss and a higher free androgen index were associated with less wrinkling. In summary, we validated known and identified additional determinants for wrinkling. Skin aging-reducing strategies should incorporate the sex differences found in this study.

INTRODUCTION

Skin aging is an ongoing process associated with declined skin function and changes in its appearance. It reflects a person's general health[1] and emotional well-being[2]. Skin aging is a complex phenotype and includes different features, of which facial wrinkles are arguably the most notable. There is large interest in understanding the pathophysiology of wrinkles because it is one of the most obvious targets for improving skin appearance and is a key anti-aging target for the cosmetic market.

Both intrinsic and extrinsic factors[3] contribute to skin aging; smoking and ultraviolet (UV) radiation are the most well known extrinsic risk factors[4,5]. High body mass index (BMI) accounts for less wrinkles[6], most probably because facial fat has an expanding/filler effect on the skin. Other determinants (associative factors) that have been linked to wrinkles include education[7], alcohol[8], and female sex steroids[9] but these findings are controversial as they have not all been replicated consistently in other studies.

The extent and characteristics of facial wrinkles differ between men and women, regarding localization and depth[10,11] and could in part be due to hormonal differences[12,13]. A possible explanation for the observed perioral skin wrinkling difference is that women have less sebaceous glands and sweat glands and a lower ratio between vessel area and connective tissue area in the dermis[14]. The impact of lifestyle and physiological factors on sex-specific skin wrinkling is not well documented.

Although three-dimensional (3D) microtopography of the dorsum of the hand as an index of actinic skin damage[15] or a digital fringe projection method to assess wrinkle severity[10] have been used, most clinical skin aging studies have used manual photonumeric scales. Most scales regard skin aging as a compound phenotype including wrinkles, telangiectasia, pigmented spots, and sagging together[3,16,17]. Therefore, it is difficult to infer the role of possible determinants specifically linked to wrinkles. Investigating the skin aging aspects separately could lead to the discovery of determinants specific for each aging phenotype. Moreover, photonumeric scales are prone to human grading bias due to scoring of different components of skin aging, for example, sagging or hair graying. A digital measure can provide a more objective, valid, and reliable measurement of skin wrinkles.

In 3831 individuals of northwest European ancestry in the Rotterdam Study (RS), we tested for associations between the main lifestyle and physiological factors and facial wrinkles in a middle-aged to elderly population[18]. This was performed in men and women separately by using digital quantification of wrinkle area measured from facial photographs.

RESULTS

Study population

Between September 2010 and June 2014, a total of 4649 participants visited the in-person examination of the RS, which includes extensive dermatological assessments. After excluding 818 individuals because of non-northwest European origin, poor image quality, make-up, and/or presence of facial hair (e.g., beards), 3831 RS participants with eligible 3D photographs were used to measure facial wrinkle area. The majority were women (N=2229; 58.2%) and the median age was 66.8 (IQR 61.2-71.9) in men and 66.4 (IQR 60.9-71.1) in women (Table 1).

Table 1. Characteristics of 3831 participants of the Rotterdam Study with 3D photographs, for the total study population and stratified by sex

Characteristic[a]	Total study population (N=3831)	Men (N=1602)	Women (N=2229)	P-value men vs. women[j]
Wrinkle area %, median [IQR]	4.0 [2.5 – 6.0]	4.5 [2.9 – 6.3]	3.6 [2.2 – 5.6]	<0.001
Age at photo in years, median [IQR]	66.5 [61.0 – 71.5]	66.8 [61.2 – 71.9]	66.4 [60.9 – 71.1]	0.069
BMI in kg/m^2, mean (SD)	27.6 (4.4)	27.6 (3.7)	27.5 (4.9)	0.360
Skin color				
pale (%)	366 (10)	134 (8)	232 (10)	0.040
white (%)	2912 (76)	1199 (75)	1713 (77)	0.160
white-to-olive (%)	553 (14)	269 (17)	284 (13)	<0.001
Smoking history[b]				
current (%)	707 (19)	339 (21)	368 (17)	<0.001
former (%)	1921 (50)	909 (57)	1012 (45)	<0.001
never (%)	1198 (31)	353 (22)	845 (38)	<0.001
Baldness[c]				
no/mild baldness (%)	2330 (61)	826 (52)	1504 (68)	<0.001
moderate (%)	874 (23)	369 (23)	505 (23)	0.920
extensive (%)	576 (15)	406 (25)	170 (8)	<0.001
Tendency to develop sunburn				
low (%)	2440 (64)	1068 (67)	1372 (62)	0.009
high (%)	1253 (33)	492 (31)	761 (34)	
Lived in sunny country[d]				
no (%)	3483 (91)	1424 (89)	2059 (92)	<0.001
yes (%)	240 (6)	144 (9)	96 (4)	
Sun-protective behavior[e]				
never/almost never (%)	1290 (34)	586 (37)	704 (32)	0.003
often/almost always/always (%)	2433 (64)	983 (61)	1450 (65)	

Table 1. Characteristics of 3831 participants of the Rotterdam Study with 3D photographs, for the total study population and stratified by sex (continued)

Characteristic[a]	Total study population (N=3831)	Men (N=1602)	Women (N=2229)	P-value men vs. women[j]
Spending winter in sunny country				
no or less than 1 month/year (%)	3434 (90)	1430 (89)	2004 (90)	0.460
yes, ≥1 month/year (%)	194 (5)	86 (5)	108 (5)	
missing (%)	203 (5)	86 (5)	117 (5)	
Outdoor work history[f]				
no (%)	1911 (50)	690 (43)	1221 (55)	<0.001
yes (%)	511 (13)	298 (19)	213 (10)	
missing (%)	1409 (37)	614 (38)	795 (36)	
Tanning bed use[g]				
never or less than 10x (%)	1944 (51)	807 (50)	1137 (51)	0.005
more than 10x (%)	325 (9)	108 (7)	217 (10)	
missing (%)	1562 (41)	687 (43)	875 (39)	
Education level[h]				
low (%)	311 (8)	108 (7)	203 (9)	0.009
medium (%)	2388 (62)	906 (57)	1482 (67)	<0.001
high (%)	1090 (29)	570 (36)	520 (23)	<0.001
Alcohol				
median use in glasses/day [IQR]	0.8 [0.1 – 1.8]	1.2 [0.3 – 2.4]	0.5 [0.1 – 1.4]	<0.001
missing (%)	626 (16)	281 (18)	345 (16)	
Dry skin presence				
no (%)	1254 (33)	588 (37)	666 (30)	<0.001
yes (%)	2573 (67)	1014 (63)	1559 (67)	
Testosterone in nmol/l, median [IQR]	na	16.7 [13.1 – 20.6]	na	na
Free androgen index[i], median [IQR]	na	na	1.3 [0.9 – 2.0]	na
missing (%)	na	na	145 (7)	na
Estradiol in pmol/l, median [IQR]	na	na	43.1 [18.4 – 74.6]	na
missing (%)	na	na	122 (6)	na

Abbreviations: BMI, body mass index; na, not applicable; SD, standard deviation.

[a]all variables have missing values <5% unless otherwise specified. Percentages are rounded to integers; [b]cigars, cigarettes, or pipe; [c]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; [d]history of living in a sunny country >1 year; [e]wearing sunglasses and/or a brimmed hat in the sunshine; [f]worked or been outdoors ≥4 hours daily during at least 25 years; [g]frequency of tanning bed visits in the past 5 years (including facial solarium); [h]low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [i]free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l); [j]t-test for normally distributed continuous data (BMI); Mann-Whitney U Test for non-normally distributed continuous data (all except BMI); chi^2 test for categorical data.

Wrinkle area measure

The distribution of the facial wrinkle area percentage was skewed towards higher values (Figure 1). The median wrinkle area percentage was higher in men than in women (men: 4.5, IQR 2.9-6.3; women: 3.6, IQR 2.2-5.6), but not for all age groups. Men had a higher wrinkle area than women in the lowest age groups (<65 years old, mean difference in wrinkle area: 1.0, P-value=2.4×10^{-17}; 65-75 years old, difference: 0.6, P-value=6.0×10^{-6}). Women had a higher wrinkle area than men in the highest age group (≥75 years old, mean difference: 0.7, P-value=0.02; Figure 2). We also found similar sex differences after stratifying for the UV variable "outdoor work history" (Supplementary Results, including Supplementary Figure S1).

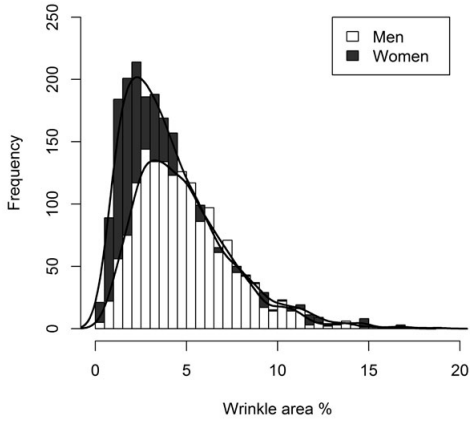

Figure 1. Distribution of the digital wrinkle area percentages split by sex. The distribution of the facial wrinkle area percentage is skewed towards higher values for both sexes. The median wrinkle area percentage was higher in men than in women.

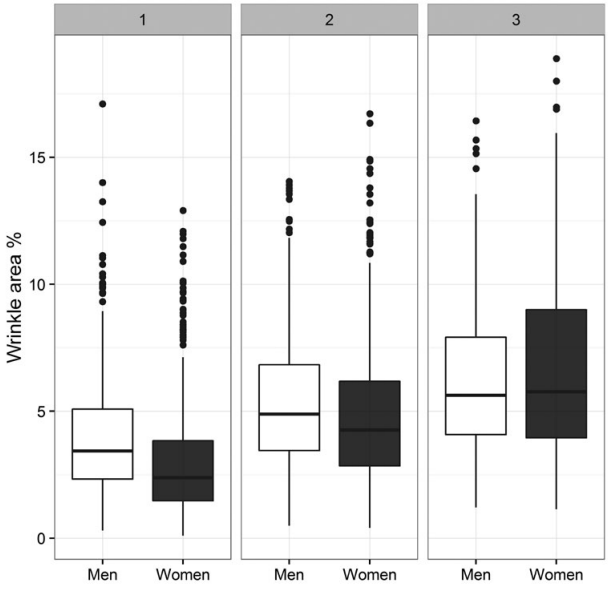

Figure 2. Boxplots of wrinkle area % per age group (1: <65 years old; 2: 65-75 years old; 3: ≥75 years old), split by sex. Men have statistically significant higher wrinkle area than women in the lowest and middle age group. Women have statistically significant higher wrinkle area than men in the highest age group.

Determinants for global facial wrinkle area

Men

We used the R^2 (coefficient of multiple determination) to calculate the percentage of wrinkle variation explained by the regression and the contribution of specific variables to the total R^2 value (see Materials and Methods). In a full model of wrinkle area (all variables included), 22% of the variability (adjusted R^2) of facial wrinkles was explained (Table 2). Age was the most important determinant of wrinkles in the linear regression analysis, as indicated by the R^2 value of 9.0% in 1602 men. Current smoking (15.5%Δ vs. never smoking) and lower BMI (1.7%Δ per unit decrease in BMI) were associated with a higher wrinkle area. Light skin color was associated with a lower wrinkle area (pale vs. white-to-olive colored skin -21.0%Δ; white vs. white-to-olive colored skin -10.3%Δ, Table 2). Testosterone levels showed a positive association with borderline significance (0.5%Δ, 95% confidence interval (CI) -0.00 to 0.94). In addition, the UV variable "spending winter in a sunny country" was borderline statistically significant (10.4%Δ, 95%CI -1.4 to 23.5) and a high susceptibility for sunburn was significantly associated with lower wrinkle area (-5.8%Δ) (Table 2). Age was nonlinearly related to wrinkle area; with increasing age, individuals showed a smaller increase in wrinkle area than younger individuals did (Supplementary Figure S2a, age^2 P-value<0.001).

Women

In 2229 women, the determinants accounted for 37.2% of the variability of facial wrinkles (Table 2). Age showed even stronger effects in wrinkle variation than in men (R^2 21.7%). Current smoking (30.9%Δ vs. never smoking) and lower BMI (1.8%Δ per unit decrease in BMI) were positively associated with wrinkling and a light skin color was inversely associated (pale vs. white-to-olive colored skin -28.5%Δ). In addition, women with a lower education level had more wrinkles compared with those with a high education level (low vs. high 16.5%Δ) and a higher alcohol intake showed a small but significant positive effect (3.9%Δ per daily glass). Extensive female baldness (-16.1%Δ vs. women with no baldness) and a higher free androgen index (FAI) (-5.6% Δ per point) showed protective effects. The nonlinear effects of age were also statistically significant in women (Supplementary Figure S2b, age^2 P-value<0.001).

As shown in Table 2, the differences in the amount of variation in wrinkles explained by the determinants (R^2 differences) between men and women were not only due to additional predictors in women but also due to the magnitude of effects of the variables significant in both groups; in women, R^2 was 0.3% higher for BMI, smoking, and skin color.

Sensitivity analyses

Two UV variables "outdoor work history" and "tanning bed use" had a high percentage of missing values: 37% and 41%, respectively, and were not included in the full model. To better test the influence of UV-exposure, we included these two variables in additional complete case analyses (N=907 men and N=1343 women). In men, outdoor work history was associated with more

Table 2. Sex-stratified multivariable linear regression of global facial wrinkle area among 1602 men and 2229 women in the Rotterdam Study

	Men (N=1602)				Women (N=2229)			
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	Adj. R^2 %	% Δ wrinkle area[a]	95% CI	P-value	Adj. R^2 %
Full model				22.0				37.2
Age				9.0				21.7
Age	11.8	[7.4 – 16.5]	<0.001		17.3	[13.0 – 21.7]	<0.001	
Age^2	-0.06	[-0.10 – -0.02]	<0.001		-0.08	[-0.10 – -0.06]	<0.001	
Resolution variation (batch)	-18.0	[-23.4 – -12.2]	<0.001	1.6	-16.5	[-22.1 – -10.5]	<0.001	0.7
Flash light variation	-0.3	[-0.4 – -0.1]	<0.001	0.8	-0.4	[-0.5 – -0.2]	<0.001	0.8
Lower BMI (per unit)	1.7	[0.9 – 2.4]	<0.001	1.0	1.8	[1.3 – 2.4]	<0.001	1.3
Skin color				0.8				1.1
pale	-21.0	[-29.5 – -11.4]	<0.001		-28.5	[-35.7 – -20.5]	<0.001	
white	-10.3	[-16.4 – -3.8]	0.002		-15.5	[-21.5 – -9.0]	<0.001	
white-to-olive	ref	ref	ref		ref	ref	ref	
Smoking history[b]				1.2				1.5
current	15.5	[6.8 – 24.8]	<0.001		30.9	[21.8 – 40.7]	<0.001	
former	-2.1	[-8.2 – 4.5]	0.529		5.7	[0.2 – 11.4]	0.042	
never	ref	ref	ref		ref	ref	ref	
Baldness[c]								0.4
no/mild baldness	ref	ref	ref		ref	ref	ref	
moderate	-5.9	[-11.9 – 0.5]	0.069		-3.9	[-9.5 – 1.9]	0.183	
extensive	-1.8	[-8.0 – 4.7]	0.577		-16.1	[-23.6 – -7.8]	<0.001	
Tendency to develop sunburn	-5.8	[-11.2 – -0.2]	0.045	0.2	-3.8	[-8.8 – 1.5]	0.159	
Lived in sunny country[d]	4.1	[-4.9 – 13.9]	0.381		-4.9	[-15.4 – 6.9]	0.399	
Sun-protective behavior[e]	0.2	[-5.0 – 5.7]	0.947		-3.8	[-8.6 – 1.4]	0.146	
Spending winter in sunny country	10.4	[-1.4 – 23.5]	0.086		2.9	[-8.0 – 15.1]	0.615	
Education level[f]								0.3
low	2.4	[-7.9 – 14.0]	0.659		16.5	[5.9 – 28.2]	0.002	
medium	2.7	[-2.8 – 8.6]	0.341		7.2	[1.0 – 13.8]	0.023	
high	ref	ref	ref		ref	ref	ref	
Alcohol (per glass/day)	0.4	[-1.2 – 2.0]	0.627		3.9	[1.7 – 6.3]	<0.001	0.4
Dry skin	-1.9	[-7.0 – 3.6]	0.497		0.8	[-4.5 – 6.3]	0.778	
Testosterone (nmol/l)	0.5	[-0.00 – 0.94]	0.050		na	na	na	
Free androgen index[g]	na	na	na		-5.6	[-7.5 – -3.7]	<0.001	1.0
Estradiol (pmol/l)	na	na	na		0.0	[0.0 – 0.0]	0.789	

The adjusted R^2 is shown for the full model and for all statistically significant variables.

Abbreviations: adj. R^2, adjusted R^2; BMI, body mass index; 95% CI, 95% confidence interval; na, not applicable; ref, reference variable.

[a]the regression betas of each determinant are presented as percentage change (%Δ) in wrinkle area (the % increase in the mean value of wrinkle area per unit increase in the independent variables), calculated by the formula: (exp^{β}-

1) * 100%; [b]cigars, cigarettes or pipe; [c]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; [d]history of living in a sunny country >1 year; [e]wearing sunglasses and/or a brimmed hat in the sunshine; [f]low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [g]free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l).

wrinkling (18.9%Δ, 95%CI 9.5–29.0), whereas in women, tanning bed frequency of ≥10 vs. <10 in the past five years was associated with more wrinkles (16.5%Δ, 95%CI 6.3–27.6, Supplementary Table S1).

Determinants for site-specific wrinkle area

Besides global facial wrinkles, site-specific wrinkle areas (i.e., crow's feet, forehead wrinkles, and in women upper lip wrinkles) were analyzed. Wrinkling around the mouth was strongly associated with smoking in women (see Supplementary Material and Supplementary Table S2 and S3 for further results) and a higher BMI was associated with greater wrinkling at the crow's feet in women.

DISCUSSION

In this large study of lifestyle and physiological factors for facial wrinkles, known associations between facial wrinkles and lower BMI, sun exposure, and smoking were replicated[4,19]. In addition, we identified protective factors including pale skin color and in women hair loss and a high FAI.

Of all variables examined, age was the largest predictor and explained most of the wrinkle variation ranging from 9.0% in men to 21.7% in women and had a larger effect per year in the middle-aged individuals than in the elderly. Fine wrinkles are regarded as an intrinsic skin aging symptom, whereas coarse wrinkles are considered a typical extrinsic skin aging symptom[20]. Our wrinkling phenotype could not distinguish between these two types of aging. The models used have captured a higher variability for wrinkles in women than in men (R^2 37.2% and 22.0%, respectively). Besides unknown/unmeasured determinants, the main unexplained variation lies in genetics; the heritability of wrinkles is estimated to be 55%[21].

Most skin aging studies have analyzed both sexes together, despite important sex differences that have been described[10,11,14,22,23]. In the sex-stratified analyses, we found that men have more wrinkles than women in the lower age groups, but in the highest age group, this is reversed – confirming earlier research[10]. Men might get more wrinkles earlier in life because of higher occupational sun exposure (e.g., from the construction industry). However, we found that the differences remained significant after adjusting the wrinkling for occupational sun exposure. Alternatively, the difference may be due to the more rapid hormonal changes occurring in postmenopausal women[24], which is supported by a Korean study demonstrating a significant relationship between

early menopause and wrinkle severity[9]. In our study, although estradiol level was not associated with wrinkles, the FAI and presence of female pattern hair loss (FPHL) showed a significant inverse association. As FPHL is, in part, related to sensitivity to testosterone levels, these findings suggest that testosterone plays a significant role in protecting women against wrinkles. Although these are interesting endocrinological findings, wrinkle-reducing strategies are unlikely to incorporate boosting testosterone levels due to undesired side effects (e.g., FPHL) in women; however, identifying the mechanism through which testosterone protects skin from wrinkling in women could help find other analogous routes to reducing wrinkling formation. Obese women have reduced sex hormone binding globulin (SHBG) levels, leading to a higher FAI and thus a relative hyperandrogenic state[25]. In our data, BMI and FAI were both statistically significant in the model together, and stratifying for BMI in women showed a persistent significant association between FAI and wrinkles in all three categories (Supplementary Results). Hence, it is unlikely FAI is only associated with wrinkles due to its relationship to BMI. In men, there is a surprising borderline significant positive effect of testosterone on wrinkling. However, we cannot completely make inferences on this association in men, as total testosterone is an inferior marker to free testosterone[26], which is not available in the RS.

The differences in skin wrinkling across sex were reflected in extent of wrinkles, associated determinants and their effect sizes. Smoking, for example, had a stronger effect in women than in men[6]. Skin color also showed a stronger effect in women. On the contrary, UV variables showed an effect in men, but not in women. Higher education previously showed an association with less wrinkles[7], but we could only confirm this in women. It could be argued that women with higher education put more effort in protecting their skin against aging-related conditions like sun damage, whereas highly educated men do not.

Strikingly, we found that light-skinned northwestern Europeans had less wrinkles than darker skinned ones. In addition, in men, the tendency to develop sunburn – which is often the case in light-skinned individuals – was associated with less wrinkles. This seems contradictive, because people with Fitzpatrick skin types[27] IV and higher have less wrinkles than those with skin types I-III[28]. However, a recent skin aging study confirmed our finding of less wrinkles in light-skinned individuals[29]. An explanation could be that light-skinned individuals are known to avoid the sun because of the UV-sensitive nature of their skin. Yet, our model also included some UV-exposure-related variables that should correct for this effect. Furthermore, light-skinned individuals in this study have already been shown to have more pigmented spots[30], which are also influenced by UV-exposure[31-33]. Alternatively, we argue that different skin types show different phenotypes of skin aging because individuals with lighter skin have atrophic skin changes manifested as fewer wrinkles and more dysplastic changes[34,35].

We found that most variables that were associated with global wrinkles were also significantly associated with specific wrinkle sites (Supplementary Table S3). Of note, smoking showed, in line with a previous study[36], a stronger association with upper lip wrinkles than with any other site or with global wrinkles. This could be the result of the high concentration of smoke around

the mouth, or the increased expression of lip lines while inhaling. Unexpectedly, BMI showed a positive association with crow's feet wrinkles in women and a similar direction but nonsignificant association in men; this finding suggests that the "filler effect" of subcutaneous fat in the crow's feet area is being counteracted by some unknown mechanism – perhaps related to the fat distribution in the face – that needs replication and further investigation.

Limitations of this study include the cross-sectional design, which precludes addressing the temporality of the observed associations. We did not measure wrinkle depth, or distinguish between fine and coarse wrinkles[20] that may have been influenced by different determinants. The assessment of UV-exposure is challenging, and the available UV variables might not have captured cumulative sun exposure accurately. In addition, sex hormones were measured in serum samples on average 5.6 years before photo-collection. This could have influenced the association with wrinkle area although it is not necessarily a limitation, because wrinkles develop over a longer period of time. There was a lack of heterogeneity in the study population – middle-aged to elderly northwestern Europeans with white skin color[37] – which reduces possible residual confounding but limits generalizability. Furthermore, although image analysis techniques were consistently applied to every image, technical variation could influence the extent of wrinkles, although we adjusted for two important technical-related variables in our models.

In conclusion, in the largest wrinkling study to date, facial skin wrinkling differs between men and women regarding the extent of wrinkling, and associated determinants and their effect sizes. This study confirmed known risk factors for facial wrinkles and discovered protectively associated factors such as light skin color in both sexes and hormonal factors such as FPHL and high FAI in women, which could help direct new prevention strategies for skin aging.

MATERIALS AND METHODS

Study population

The Rotterdam Study (RS) is an ongoing prospective population-based cohort study following 14,926 participants aged ≥45 years in Ommoord, a suburb of Rotterdam in the Netherlands, since 1990. Details of the study design and objectives have been described elsewhere[18]. The RS has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sports of the Netherlands, implementing the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)". All participants provided written informed consent to participate in the study.

Since 2010, skin examinations have been conducted by trained physicians, focusing on the most common skin diseases. In addition, standardized high-resolution digital 3D facial photographs (Premier 3dMDface3-plus UHD, Atlanta, GA, USA) are being collected. Between September 2010 and June 2014, a total of 4649 participants have been photographed and examined at the research center.

Wrinkles

High-resolution standardized full face photographs were obtained in a room without daylight. Details of digital image acquisition and its validation have been described elsewhere[38] and in the Supplementary Material. Enface 2D photographs were exported from the 3D images and masked to isolate the skin areas in the image using semi automated masking (MATLAB, The MathWorks, Inc, Natick, MA, USA, version 2013a). Using MATLAB, wrinkle area was calculated from the 2D facial images, expressed as a percentage of the facial skin area (Figure 3).

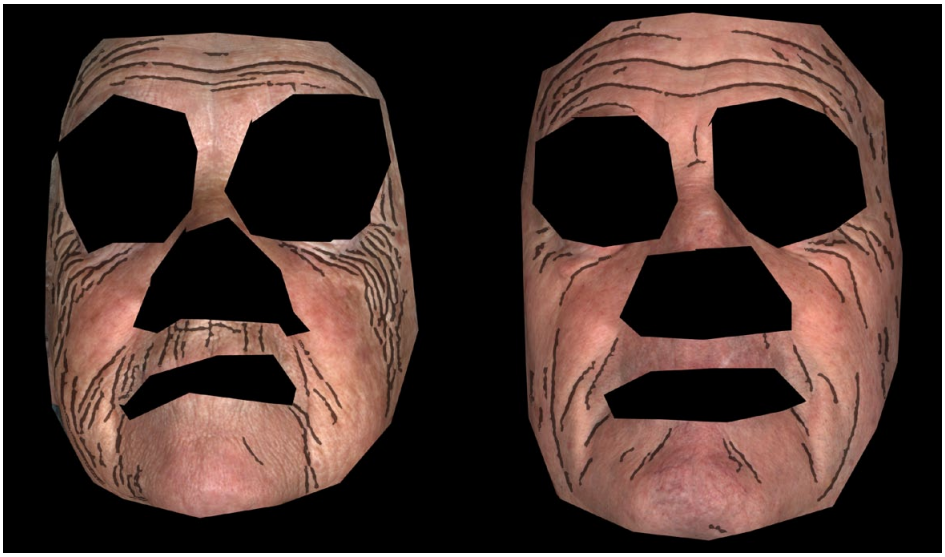

Figure 3. Digital extraction of wrinkles. Non-skin parts (i.e., eyes, nostrils, and mouth) are masked and wrinkle area is measured in units of pixels. Left: female participant; right: male participant.

Besides measuring wrinkles of the whole face, we also measured wrinkle area at three specific sites (crow's feet, forehead wrinkles, and in women upper lip wrinkles) that was readily available for a subset of participants, see Supplementary Methods and Results.

Determinants

Sex and age (in years) at date of photo were retrieved from the database. Information on other physiological and lifestyle factors was collected by interview, physical examination, and blood serum measurements. In the interview, variables on level of education, smoking habit and UV-related questions were collected[18]. Variables collected by physical examination at the research center were BMI (calculated by dividing weight by the squared height), presence of dry skin, baldness, and constitutional skin color assessed at the sun-unexposed skin of the upper body, that is, abdomen and inner upper arm: pale, white, and white-to-olive, as validated by a previous study[37]. Baldness was based on the Norwood-Hamilton scale[39,40] for men and the Ludwig scale[41]

for women. For the analyses, we classified these scales into three categories of male baldness: none or minimal (Norwood-Hamilton score 1, 2, 3, 9, 10, 11), moderate (Norwood-Hamilton score 4, 5, 6, 12), and extensive baldness (Norwood-Hamilton score 7, 8). Female baldness was also classified into three categories, that is, none or minimal (scored as "none"), moderate (Ludwig scale score 1), and extensive baldness (Ludwig scale score 2 and 3). In serum samples, estradiol, testosterone, and sex hormone binding globulin (SHBG) were measured on average 5.6 years before photo-collection. The free androgen index (FAI) was calculated for women, using the formula: (total testosterone / SHBG * 100)[42]. Details of all variables used are described in the Supplementary Methods.

Statistical analysis

We compared characteristics between men and women using chi^2 tests for categorical variables, independent samples t-tests for normally distributed continuous variables and Mann-Whitney U tests for non-normally distributed continuous variables.

For the main association analyses, we excluded variables with >35% missing values, namely the UV variables "outdoor work history" and "tanning bed use". For the other missing values (maximum missing data per variable was 17.5%), we performed multiple imputation based on all available variables shown in Table 1. This was performed using the Multivariate Imputation by Chained Equations (MICE) software package in R (http://www.R-project.org), with an iteration of 20.

To investigate the associations between potential determinants and wrinkle area, we used linear regressions. To correct for technical variation, we included two variables which accounted for possible variations in resolution and flash light in all regression models. For variations in resolution, a variable describing the batch number was used. For flash light variation, the in-person difference between skin lightness in the images and that taken by a spectrophotometer (CM-600d; Konica-Minolta, Osaka, Japan) on the cheek was used, by calculating the residuals of these two lightness variables regressed on each other[30].

The residuals of the linear regression of wrinkle area did not fit a normal distribution. Therefore, wrinkle area was transformed using the natural logarithm (ln). This resulted in an approximately normal distribution of the residuals of the regression. The resulting effect estimates (regression betas) were transformed using the formula: $(exp^{\beta}-1) * 100\%$ and can be interpreted as the percentage difference (%Δ): the percentage increase in the mean value of wrinkle area per unit increase of a determinant, for example, 3% increase in wrinkle area per 1 year of age.

We found statistically significant interactions between sex and four other variables (age, smoking, skin color, and education – Supplementary Methods, including Supplementary Table S4). Therefore, we stratified by sex in all analyses. Interactions were also tested for all plausible pairs of variables in men and women separately (Supplementary Methods) but none of these were significant (data not shown).

There was a non-linear relationship between age and wrinkle area (Supplementary Figure S2). Therefore, we added the squared term "age^2" to the analyses. Besides age, age^2, and the two technical variables, we added other variables to create a fully adjusted multivariable linear regression analysis. Variables were selected based on known literature and biologically plausible associations. In addition, we investigated the contribution of each variable to the model, by calculating the difference between the adjusted R^2 of the full model with and without each variable of interest (Supplementary Material).

Sensitivity analyses

The two UV variables that represented UV-exposure the best were "outdoor work history" and "tanning bed use". However, these variables had a high percentage of missing values (37% and 41%, respectively) and therefore were not included in the full model. We performed sensitivity analyses including complete cases of these two variables. For incomplete data of the other variables, we performed multiple imputation as mentioned above. Besides the two UV variables, the analyses included the same variables as the main analyses.

All analyses were performed using SPSS for Windows version 21.0 (SPSS, Chicago, IL) and software package R (http://www.R-project.org). A two-sided P-value of <0.05 was considered statistically significant.

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SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

Digital image acquisition

Details of digital image acquisition have been described elsewhere[1]. In short, high resolution standardized full face photographs were obtained with a Premier 3dMD face3-plus UHD camera (3dMD, Atlanta, GA, USA), in a room without daylight. Participants were asked not to wear any make-up, facial cream, or jewelry. Three 2D photographs (2452×2056 pixels, 14.7MB in BMP format) were taken simultaneously (one upper-frontal and two 45° lateral photos) in uniform surroundings, and by combining these, the 3dMD software rendered a 3D facial image. The machine was calibrated daily to control for camera position and environmental light intensity. Images were obtained under the same conditions, apart from a lighting change halfway through the study, which was accounted for in the analyses. The distance between the subject and the cameras was fixed but slight variation cannot be excluded due to the lack of a face rest. The raw files from the 3DMD system were further processed to regenerate 2D frontal images (1920×1080 pixels) of the whole face.

Wrinkle area global facial wrinkles

Using MATLAB, wrinkle area was calculated from the 2D facial images, expressed as a percentage of the facial skin area. Details of these measurements have recently been published[1]. In short, based on the contrast in color and lightening, the amount of pixels detected as wrinkles divided by the amount of pixels of the whole face resulted in the relative wrinkle area. This was multiplied by 100 to create the facial wrinkle percentage.

Wrinkle area site-specific facial wrinkles

Besides measuring wrinkles of the whole face, we have also measured wrinkles at three specific sites (crow's feet, forehead wrinkles, and in women upper lip wrinkles). The technical wrinkle measurements were the same as for the global facial wrinkles, however these measurements were performed on the original 2D photographs of the left side of the face[1].

Determinants

Information on patient characteristics and lifestyle factors were collected by interview (level of education, smoking and alcohol habits, UV-related questions) and physical examination[2]. Level of education was assessed during the interview, classified into three categories, i.e. low (primary education with or without a higher not completed education), medium (lower secondary education, lower vocational education, and intermediate vocational education), and high (general secondary education, higher vocational education, and university). Smoking habit was transformed into

current, former and never; alcohol habit was shown as glasses per day. In addition, six variables used as proxy for UV-exposure were available from interview data: tendency to develop sunburn (low vs. high), history of more than 25 years of outdoor work (yes vs. no), having wintered in a sunny country between September and May for at least one month during the past 5 years (yes vs. no), having lived in a sunny country for more than 1 year (yes vs. no), sun protective behavior (i.e. wearing sunglasses and/or a brimmed hat in the sun categorized into never/almost never vs. often/almost always/always) and use of tanning beds (fewer vs. more than ten times in the last five years). Body mass index (BMI) was measured at the research center and calculated by dividing weight (in kg) by the squared height (in m).

Other determinants were scored during full body skin examination by trained physicians: presence of dry skin (binary response), skin color (pale, white, and white-to-olive)[3] and baldness based on the Norwood-Hamilton scale[4,5] for men and the Ludwig scale[6] for women. For the analyses, we classified these scales into three categories of male baldness: none or minimal (Norwood-Hamilton score 1, 2, 3, 9, 10, 11), moderate (Norwood-Hamilton score 4, 5, 6, 12), and extensive baldness (Norwood-Hamilton score 7, 8). Female baldness was also classified into three categories, i.e. none or minimal (scored as "none"), moderate (Ludwig scale score 1), and extensive baldness (Ludwig scale score 2 and 3). Furthermore, estradiol (in pmol/l), testosterone (in nmol/l) and sex hormone binding globulin (SHBG, in nmol/l) were measured in serum samples on average 5.6 years before photo-collection. Fasting blood samples were drawn in the morning (≤11 am). Estradiol levels were measured with a radioimmunoassay and SHBG with the Immulite platform (Diagnostics Products Corporation Breda). The corresponding intra- and interassay coefficients of variation with lower limit of detection of the assays were less than 11%, less than 11%, and 18.35 pmol/l for estradiol and less than 4%, less than 5%, and 0.02 nmol/l for SHBG. Serum levels of testosterone were measured with liquid chromatography-tandem mass spectrometry, with a corresponding interassay coefficient of variation of less than 5% and a lower limit of quantification of 0.07 nmol/l. The free androgen index (FAI) was calculated for women, using the formula: (total testosterone / SHBG) * 100 [7].

Statistical analysis

The phenotype (digital global wrinkle area) followed a right-skewed distribution. In order to meet the criteria for performing a linear regression, we ln-transformed the phenotype, resulting in an approximately normal distribution of both the phenotype and the residuals of the regression.

The site-specific wrinkles also followed a right-skewed distribution, but also included a considerable proportion of zeros as value for some photos, indicating there were no wrinkles at that site at all. Therefore, we could not ln-transform the phenotype. To better fit the data for regression, we used rank-based inverse normal transformation, where the mean is set to zero and the standard deviation to one[8].

We investigated statistical interaction between sex and other covariates. We added each potential interaction term to the final model one at a time to investigate its significance, namely:

sex*age, sex*BMI, sex*smoking, sex*skin color, sex*baldness, sex*alcohol, sex*education, and sex*tendency to develop sunburn. Statistical interaction between sex and the following variables was observed after adjusting for other available covariates: smoking (P-value 0.001 and 0.003), age (P-value<0.001), skin color (P-value 0.49 and 0.045) and education (P-value 0.006 and 0.003), Supplementary Table S4. Further analyses were therefore stratified per sex.

We also investigated statistical interaction in men and women separately and found no significant interactions; the following interaction terms were tested: smoking*each UV variable, smoking*BMI, smoking*age, smoking*alcohol, smoking*education, skin color*each UV variable, BMI*education, and BMI*alcohol.

The adjusted R^2 attributable to each variable was calculated by omitting each variable from the final model one by one and calculating the adjusted R^2 of these models. These were subtracted from the adjusted R^2 of the full model. The adjusted R^2 takes into account the number of variables added to the model. The adjusted R^2 of age was calculated for the terms age and age^2 taken together.

Sensitivity analyses

We have investigated the role of FAI in wrinkles for different categories of BMI in women. We created three groups: BMI<25 kg/m^2; BMI 25-30 kg/m^2; BMI>30 kg/m^2. We performed linear regression analyses for the complete cases in these three groups separately, including all variables mentioned in Table 1.

RESULTS

Global facial wrinkles – sensitivity analyses

To investigate whether the sex differences in wrinkle area found within different age groups (Figure 2 main manuscript) were related to a differential UV-exposure (e.g. occupational exposure) we have also performed the analyses correcting for the UV variable “outdoor work history”, by stratifying for this variable (Supplementary Figure S1). Only complete cases of the variable “outdoor work history” were used for this analysis, N=2422.

For the group without occupational UV-exposure, men had significantly more wrinkling in the age group <65 years old (independent samples t-test, P-value 1.4×10^{-9}) and 65-75 years old (P-value 3.7×10^{-3}); women had significantly more wrinkling in the highest age group of ≥75 years old (P-value 1.8×10^{-2}). For the group with occupational UV-exposure, men had significantly more wrinkling in the age group <65 years old (P-value 9.1×10^{-10}). Men also had a trend towards more wrinkling in the age group 65-75 years old, but this was not significant (P-value 0.15). For the highest age group ≥75 years old, women had more wrinkles than men did, but this was also not significant (P-value 0.37). Most probably, the last-mentioned groups were too small (due to stratifying for 3 different variables: sex, age category and occupational exposure) to have enough

power to show a significant effect; in the group with occupational UV-exposure there were 98 men and 73 women aged 65-75 years old and 34 men and 12 women aged ≥75 years old.

To further investigate the relationship between FAI and wrinkles in women, we have stratified women in three groups by BMI category. The association of FAI with wrinkles remained significant in all three groups (multiple linear regression of complete cases, including all variables mentioned in Table 1): -11.6%Δ, 95%CI -17.3 to -5.6 in women with BMI<25 kg/m^2, N=517; -3.9%Δ, 95%CI -6.5 to -1.1 in women with BMI 25-30 kg/m^2, N=633; -6.7%Δ, 95%CI -10.8 to -2.4 in women with BMI>30 kg/m^2, N=392.

Site-specific facial wrinkles

The correlations between crow's feet wrinkles, forehead wrinkles and upper lip wrinkles vs. global facial wrinkles were similar (0.48 – 0.58, Supplementary Table S2). Correlations between different facial sites were not high (0.23 – 0.29), indicating that there might be variation in the effect sizes of the determinants for each different site. As shown in Supplementary Table S3a-c we found that most determinants associated with global wrinkles were also associated with site-specific wrinkles. Age remained significantly associated with all wrinkle sites. BMI remained significantly inversely associated with forehead wrinkles and women's upper lip wrinkles, but was positively associated with crow's feet. Light skin color remained inversely associated with crow's feet in both sexes and with forehead wrinkles in women. Smoking was associated with crow's feet in men and with forehead wrinkles and upper lip wrinkles in women. The association of smoking with upper lip wrinkles was higher than that with global wrinkles or any other wrinkle site. Free androgen index in women remained inversely associated with forehead and upper lip wrinkles.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1. Sex-stratified multivariable linear regression results of global facial wrinkle area: sensitivity analysis, containing all variables from Table 1 for complete cases (907 men and 1343 women in the RS), including the UV variables "outdoor work history" and "tanning bed use"

	Men (N=907)			Women (N=1343)		
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	% Δ wrinkle area[a]	95% CI	P-value
Age	15.8	[7.8 – 24.4]	<0.001	15.9	[9.1 – 23.1]	<0.001
Age^2	-0.1	[-0.15 – -0.03]	0.001	-0.07	[-0.1 – -0.03]	0.002
Resolution variation (batch)	-28.3	[-42.9 – -10.0]	0.004	-20.5	[-32.9 – -5.9]	0.008
Flash light variation	-0.24	[-0.42 – -0.06]	0.008	-0.4	[-0.55 – -0.20]	<0.001
Lower BMI (per unit)	1.5	[0.6 – 2.5]	0.002	2.0	[1.2 – 2.7]	<0.001
Skin color						
pale	-22.8	[-32.9 – -11.2]	<0.001	-29.5	[-38.3 – -19.5]	<0.001
white	-10.4	[-18.8 – -1.2]	0.027	-19.9	[-27.5 – -11.5]	<0.001
white-to-olive	ref	ref	ref	ref	ref	ref
Smoking history[b]						
current	15.6	[4.6 – 27.8]	0.005	33.4	[21.3 – 46.7]	<0.001
former	-2.6	[-10.8 – 6.2]	0.549	5.1	[-2.4 – 13.3]	0.189
never	ref	ref	ref	ref	ref	ref
Baldness[c]						
no/mild baldness	ref	ref	ref	ref	ref	ref
moderate	-3.7	[-12.5 – 6.1]	0.452	-3.6	[-11.5 – 4.9]	0.395
extensive	-0.8	[-9.8 – 9.2]	0.874	-21.8	[-32.6 – -9.1]	0.001
Tendency to develop sunburn	-5.4	[-12.8 – 2.5]	0.175	0.1	[-7.0 – 7.8]	0.973
Lived in sunny country[d]	2.4	[-11.6 – 18.6]	0.749	-3.5	[-16.9 – 12.1]	0.642
Sun-protective behavior[e]	-0.1	[-7.2 – 7.5]	0.978	-6.6	[-13.0 – 0.3]	0.060
Spend winter in sunny country	10.9	[-6.9 – 32.2]	0.246	1.2	[-13.9 – 18.9]	0.890
Outdoor work history[f]	18.9	[9.5 – 29.0]	<0.001	3.5	[-5.6 – 13.5]	0.461
Tanning bed use[g]	6.0	[-5.1 – 18.3]	0.303	16.5	[6.3 – 27.6]	0.001
Education level[h]						
low	-7.4	[-20.1 – 7.3]	0.307	13.6	[0.4 – 28.5]	0.044
medium	-1.5	[-9.0 – 6.6]	0.712	8.5	[0.6 – 17.1]	0.036
high	ref	ref	ref	ref	ref	ref
Alcohol (per glass/day)	0.6	[-1.7 – 2.9]	0.619	3.7	[0.5 – 7.0]	0.025
Dry skin	-3.3	[-10.0 – 3.8]	0.351	-0.7	[-7.2 – 6.3]	0.844
Testosterone (nmol/l)	0.44	[-0.19 – 1.07]	0.172	na	na	na
Free androgen index[i]	na	na	na	-8.2	[-11.0 – -5.2]	<0.001
Estradiol (pmol/l)	na	na	na	0.0	[-0.02 – 0.02]	0.542

Abbreviations: BMI, body mass index; 95% CI, 95% confidence interval; na, not applicable; ref, reference variable; RS, Rotterdam Study.

[a]the regression betas of each determinant are presented as percentage change (%Δ) in wrinkle area (the % increase in the mean value of wrinkle area per unit increase in the independent variables), calculated by the formula: (exp^{β}-1) * 100%; [b]cigars, cigarettes or pipe; [c]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; [d]history of living in a sunny country >1 year; [e]wearing sunglasses and/or a brimmed hat in the sunshine; [f]worked or been outdoors ≥ 4 hours daily during at least 25 years; [g]frequency of tanning bed visits in the past 5 years (including facial solarium): more than 10x vs. never or less than 10x; [h]low (primary education); medium (lower secondary education/lower vocational education/ intermediate vocational education); high (general secondary education/higher vocational education/university); [i]free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l).

Supplementary Table S2. Spearman's correlation coefficients for global facial wrinkles and site-specific facial wrinkles

	Global wrinkles	Crow's feet wrinkles	Forehead wrinkles
Crow's feet wrinkles	0.48[a]	-	-
Forehead wrinkles	0.58[b]	0.29[d]	-
Upper lip wrinkles	0.52[c]	0.28[e]	0.23[f]

All correlation coefficients have P-values <0.001.

[a]N=3299; [b]N=3261; [c]N=1153 women; [d]N=3261; [e]N=1078 women; [f]N=1068 women.

Supplementary Table S3a. Sex-stratified multivariable linear regression results of crow's feet wrinkle area compared to global facial wrinkle area in the Rotterdam Study

	Global wrinkles Men (N=1602)			Global wrinkles Women (N=2229)			Crow's feet wrinkles Men (N=1370)		Crow's feet wrinkles Women (N=1929)	
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	% Δ wrinkle area[a]	95% CI	P-value	Beta	P-value	Beta	P-value
Age										
age	11.8	[7.4 – 16.5]	<0.001	17.3	[13.0 – 21.7]	<0.001	0.27	<0.001	0.14	<0.001
age^2	-0.06	[-0.10 – -0.02]	<0.001	-0.08	[-0.10 – -0.06]	<0.001	-0.002	<0.001	0.00	<0.001
Batch										
batch 1	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
batch 2	-18.0	[-23.4 – -12.2]	<0.001	-16.5	[-22.1 – -10.5]	<0.001	0.05	0.50	0.04	0.522
batch 3	na	na	na	na	na	na	0.17	0.06	0.09	0.206
Flash light variation	-0.3	[-0.4 – -0.1]	<0.001	-0.4	[-0.5 – -0.2]	<0.001	-0.014	<0.001	0.00	0.016
Lower BMI (per unit)	1.7	[0.9 – 2.4]	<0.001	1.8	[1.3 – 2.4]	<0.001	-0.012	0.11	-0.01	0.004
Skin color										
pale	-21.0	[-29.5 – -11.4]	<0.001	-28.5	[-35.7 – -20.5]	<0.001	-0.20	0.09	-0.22	0.019
white	-10.3	[-16.4 – -3.8]	0.002	-15.5	[-21.5 – -9.0]	<0.001	-0.17	0.02	-0.11	0.105
white-to-olive	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Smoking history[b]										
current	15.5	[6.8 – 24.8]	<0.001	30.9	[21.8 – 40.7]	<0.001	0.27	<0.001	0.01	0.934
former	-2.1	[-8.2 – 4.5]	0.529	5.7	[0.2 – 11.4]	0.042	0.038	0.58	-0.06	0.235
never	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Baldness[c]										
no/mild baldness	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
moderate	-5.9	[-11.9 – 0.5]	0.069	-3.9	[-9.5 – 1.9]	0.183	0.11	0.13	0.06	0.239
extensive	-1.8	[-8.0 – 4.7]	0.577	-16.1	[-23.6 – -7.8]	<0.001	0.056	0.41	-0.06	0.511
Tendency to develop sunburn	-5.8	[-11.2 – -0.2]	0.045	-3.8	[-8.8 – 1.5]	0.159	-0.031	0.61	-0.05	0.257

Supplementary Table S3a. Sex-stratified multivariable linear regression results of crow's feet wrinkle area compared to global facial wrinkle area in the Rotterdam Study (continued)

	Global wrinkles Men (N=1602)			Global wrinkles Women (N=2229)			Crow's feet wrinkles Men (N=1370)		Crow's feet wrinkles Women (N=1929)	
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	% Δ wrinkle area[a]	95% CI	P-value	Beta	P-value	Beta	P-value
Lived in sunny country[d]	4.1	[-4.9 – 13.9]	0.381	-4.9	[-15.4 – 6.9]	0.399	0.16	0.10	-0.06	0.532
Sun-protective behavior[e]	0.2	[-5.0 – 5.7]	0.947	-3.8	[-8.6 – 1.4]	0.146	-0.006	0.91	0.01	0.821
Spend winter in sunny country	10.4	[-1.4 – 23.5]	0.086	2.9	[-8.0 – 15.1]	0.615	-0.026	0.83	-0.05	0.646
Education level[f]										
low	2.4	[-7.9 – 14.0]	0.659	16.5	[5.9 – 28.2]	0.002	0.25	0.02	-0.04	0.626
medium	2.7	[-2.8 – 8.6]	0.341	7.2	[1.0 – 13.8]	0.023	0.14	0.02	0.04	0.415
high	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Alcohol (per glass/day)	0.39	[-1.2 – 2.0]	0.627	3.9	[1.7 – 6.3]	<0.001	0.022	0.22	0.02	0.243
Dry skin	-1.9	[-7.0 – 3.6]	0.497	0.8	[-4.5 – 6.3]	0.778	0.002	0.97	0.01	0.901
Testosterone (nmol/l)	0.47	[-0.00 – 0.94]	0.050	na	na	na	0.0001	0.99	na	na
Free androgen index[g]	na	na	na	-5.6	[-7.5 – -3.7]	<0.001	na	na	-0.02	0.151
Estradiol (pmol/l)	na	na	na	0.0	[0.0 – 0.0]	0.789	na	na	0.00	0.663

Abbreviations: BMI, body mass index; 95% CI, 95% confidence interval; na, not applicable; ref, reference variable.

[a]the regression betas of each determinant are presented as percentage change (%Δ) in wrinkle area (the % increase in the mean value of wrinkle area per unit increase in the independent variables), calculated by the formula: $(\exp^{\beta}-1) * 100\%$; [b]cigars, cigarettes or pipe; [c]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; [d]history of living in a sunny country >1 year; [e]wearing sunglasses and/or a brimmed hat in the sunshine; [f]low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [g]free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l).

Supplementary Table S3b. Sex-stratified multivariable linear regression results of forehead wrinkle area compared to global facial wrinkle area in the Rotterdam Study

	Global wrinkles Men (N=1602)			Global wrinkles Women (N=2229)			Forehead wrinkles Men (N=1351)		Forehead wrinkles Women (N=1910)	
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	% Δ wrinkle area[a]	95% CI	P-value	Beta	P-value	Beta	P-value
Age										
age	11.8	[7.4 – 16.5]	<0.001	17.3	[13.0 – 21.7]	<0.001	0.01	0.0012	0.03	<0.001
age^2	-0.06	[-0.10 – -0.02]	<0.001	-0.08	[-0.10 – -0.06]	<0.001	na	na	na	na
Batch										
batch 1	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
batch 2	-18.0	[-23.4 – -12.2]	<0.001	-16.5	[-22.1 – -10.5]	<0.001	0.04	0.5787	0.18	0.018
batch 3	na	na	na	na	na	na	0.17	0.0442	0.16	0.076
Flash light variation	-0.3	[-0.4 – -0.1]	<0.001	-0.4	[-0.5 – -0.2]	<0.001	0.00	0.201	0.00	0.165
Lower BMI (per unit)	1.7	[0.9 – 2.4]	<0.001	1.8	[1.3 – 2.4]	<0.001	0.02	0.0104	0.02	0.001
Skin color										
pale	-21.0	[-29.5 – -11.4]	<0.001	-28.5	[-35.7 – -20.5]	<0.001	-0.06	0.5671	-0.41	<0.001
white	-10.3	[-16.4 – -3.8]	0.002	-15.5	[-21.5 – -9.0]	<0.001	-0.05	0.4465	-0.30	<0.001
white-to-olive	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Smoking history[b]										
current	15.5	[6.8 – 24.8]	<0.001	30.9	[21.8 – 40.7]	<0.001	0.04	0.6026	0.27	<0.001
former	-2.1	[-8.2 – 4.5]	0.529	5.7	[0.2 – 11.4]	0.042	-0.05	0.4061	0.03	0.635
never	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Baldness[c]										
no/mild baldness	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
moderate	-5.9	[-11.9 – 0.5]	0.069	-3.9	[-9.5 – 1.9]	0.183	-0.03	0.6283	0.06	0.370
extensive	-1.8	[-8.0 – 4.7]	0.577	-16.1	[-23.6 – -7.8]	<0.001	0.07	0.2703	-0.12	0.278
Tendency to develop sunburn	-5.8	[-11.2 – -0.2]	0.045	-3.8	[-8.8 – 1.5]	0.159	-0.10	0.1017	0.01	0.817

Supplementary Table S3b. Sex-stratified multivariable linear regression results of forehead wrinkle area compared to global facial wrinkle area in the Rotterdam Study (continued)

	Global wrinkles Men (N=1602)			Global wrinkles Women (N=2229)			Forehead wrinkles Men (N=1351)		Forehead wrinkles Women (N=1910)	
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	% Δ wrinkle area[a]	95% CI	P-value	Beta	P-value	Beta	P-value
Lived in sunny country[d]	4.1	[-4.9 – 13.9	0.381	-4.9	[-15.4 – 6.9]	0.399	0.19	0.0367	-0.04	0.722
Sun-protective behavior[e]	0.2	[-5.0 – 5.7]	0.947	-3.8	[-8.6 – 1.4]	0.146	-0.02	0.7474	-0.04	0.508
Spend winter in sunny country	10.4	[-1.4 – 23.5]	0.086	2.9	[-8.0 – 15.1]	0.615	0.30	0.0121	-0.11	0.336
Education level[f]										
low	2.4	[-7.9 – 14.0]	0.659	16.5	[5.9 – 28.2]	0.002	0.07	0.5011	0.19	0.066
medium	2.7	[-2.8 – 8.6]	0.341	7.2	[1.0 – 13.8]	0.023	0.03	0.6164	-0.02	0.780
high	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Alcohol (per glass/day)	0.39	[-1.2 – 2.0]	0.627	3.9	[1.7 – 6.3]	<0.001	-0.02	0.3571	-0.02	0.469
Dry skin	-1.9	[-7.0 – 3.6]	0.497	0.8	[-4.5 – 6.3]	0.778	-0.05	0.3853	-0.01	0.889
Testosterone (nmol/l)	0.47	[-0.00 – 0.94]	0.050	na	na	na	0.00	0.5578	na	na
Free androgen index[g]	na	na	na	-5.6	[-7.5 – -3.7]	<0.001	na	na	-0.06	0.005
Estradiol (pmol/l)	na	na	na	0.0	[0.0 – 0.0]	0.789	na	na	0.00	0.892

Age^2 was not added in the model for forehead wrinkles as it was not statistically significant.

Abbreviations: BMI, body mass index; 95% CI, 95% confidence interval; na, not applicable; ref, reference variable.

[a]the regression betas of each determinant are presented as percentage change (%Δ) in wrinkle area (the % increase in the mean value of wrinkle area per unit increase in the independent variables), calculated by the formula: ($\exp^{\beta}$-1) * 100%; [b]cigars, cigarettes or pipe; [c]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; [d]history of living in a sunny country >1 year; [e]wearing sunglasses and/or a brimmed hat in the sunshine; [f]low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [g]free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l).

Supplementary Table S3c. Multivariable linear regression results of upper lip wrinkle area in women compared to global facial wrinkle area in women in the Rotterdam Study

	Global wrinkles Women (N=2229)			Upper lip wrinkles Women (N=1153)	
Characteristic	% Δ wrinkle area[a]	95% CI	P-value	Beta	P-value
Age					
age	17.3	[13.0 – 21.7]	<0.001	0.13	0.001
age^2	-0.08	[-0.10 – -0.06]	<0.001	0.00	0.033
Batch					
batch 1	ref	ref	ref	ref	ref
batch 2	-16.5	[-22.1 – -10.5]	<0.001	-0.10	0.178
Flash light variation	-0.4	[-0.5 – -0.2]	<0.001	0.00	0.720
Lower BMI (per unit)	1.8	[1.3 – 2.4]	<0.001	0.02	0.001
Skin color					
pale	-28.5	[-35.7 – -20.5]	<0.001	0.14	0.187
white	-15.5	[-21.5 – -9.0]	<0.001	0.05	0.535
white-to-olive	ref	ref	ref	ref	ref
Smoking history[b]					
current	30.9	[21.8 – 40.7]	<0.001	0.45	<0.001
former	5.7	[0.2 – 11.4]	0.042	0.13	0.019
never	ref	ref	ref	ref	ref
Baldness[c]					
no/mild baldness	ref	ref	ref	ref	ref
moderate	-3.9	[-9.5 – 1.9]	0.183	-0.05	0.385
extensive	-16.1	[-23.6 – -7.8]	<0.001	-0.18	0.066
Tendency to develop sunburn	-3.8	[-8.8 – 1.5]	0.159	0.02	0.768
Lived in sunny country[d]	-4.9	[-15.4 – 6.9]	0.399	-0.10	0.423
Sun-protective behavior[e]	-3.8	[-8.6 – 1.4]	0.146	0.08	0.144
Spend winter in sunny country	2.9	[-8.0 – 15.1]	0.615	-0.18	0.124
Education level[f]					
low	16.5	[5.9 – 28.2]	0.002	0.11	0.289
medium	7.2	[1.0 – 13.8]	0.023	0.19	0.001
high	ref	ref	ref	ref	ref
Alcohol (per glass/day)	3.9	[1.7 – 6.3]	<0.001	-0.02	0.507
Dry skin	0.8	[-4.5 – 6.3]	0.778	0.04	0.395
Free androgen index[g]	-5.6	[-7.5 – -3.7]	<0.001	-0.05	0.035
Estradiol (pmol/l)	0.0	[0.0 – 0.0]	0.789	0.00	0.572

Abbreviations: BMI, body mass index; 95% CI, 95% confidence interval; ref, reference variable.

[a]the regression betas of each determinant are presented as percentage change (%Δ) in wrinkle area (the % increase in the mean value of wrinkle area per unit increase in the independent variables), calculated by the formula: ($\exp^{\beta}$-1) * 100%; [b]cigars, cigarettes or pipe; [c]based on the Ludwig scale for women; None or minimal: Ludwig scale score none. Moderate: Ludwig scale score 1. Extensive: Ludwig scale score 2, 3; [d]history of living in a sunny country >1 year; [e]wearing sunglasses and/or a brimmed hat in the sunshine; [f]low (primary education); medium (lower second-

ary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [g]free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l).

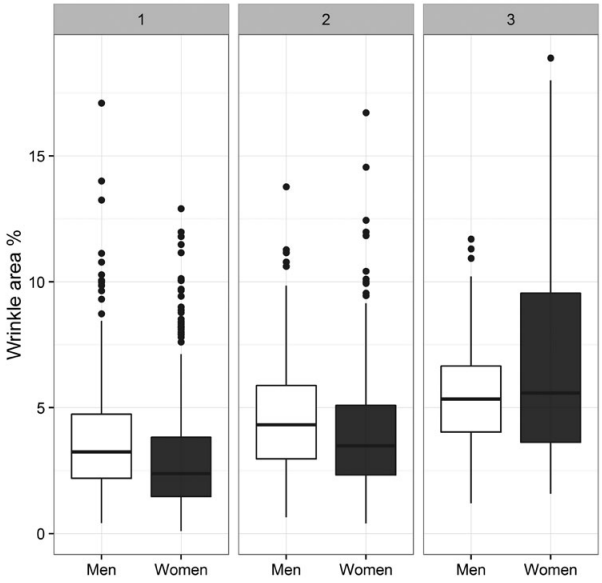
Supplementary Table S4. Betas and P-values for each interaction term, added to the final fully adjusted linear regression model available for both sexes (N=3019; only complete cases used)

Interaction term	Beta	P-value
Sex*age	-2.2×10^{-2}	<0.001
Sex*BMI	-3.6×10^{-4}	0.95
Sex*smoking history[a]		
current	-0.19	3.3×10^{-3}
former	-0.16	1.1×10^{-3}
never	ref	ref
Sex*skin color		
very white	0.18	4.5×10^{-2}
white	0.04	0.49
white-to-olive	ref	ref
Sex*baldness[b]		
no/mild baldness	ref	ref
moderate	-9.0×10^{-2}	7.5×10^{-2}
extensive	6.6×10^{-2}	0.29
Sex*alcohol (per glass/day)	-2.6×10^{-2}	7.6×10^{-2}
Sex*education level[c]		
low	-0.25	2.7×10^{-3}
medium	-0.13	6.3×10^{-3}
high	ref	ref
Sex*tendency to develop sunburn	1.8×10^{-2}	0.68

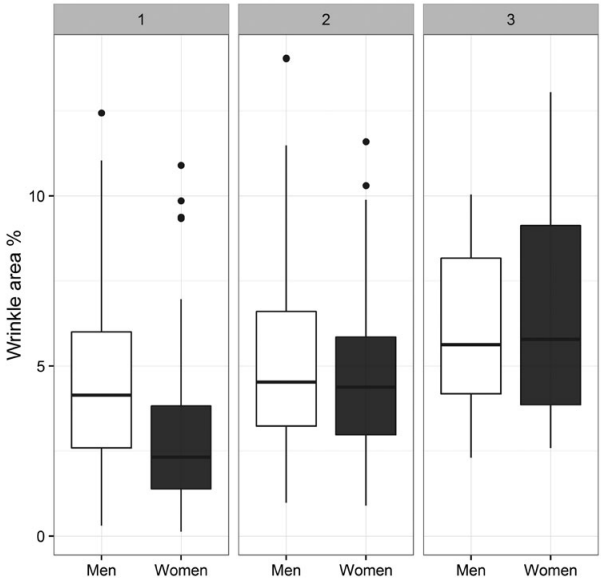
Abbreviation: ref, reference variable.

[a]smoking history: cigars, cigarettes or pipe; [b]baldness: 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; [c]education level: low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university).

A

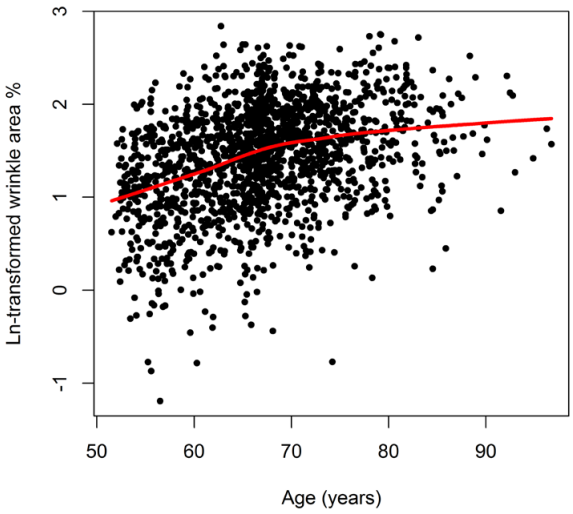


B



Supplementary Figure S1. Boxplots of wrinkle area percentage per age group (1: <65 years old; 2: 65-75 years old; 3: ≥75 years old), split by sex and stratified by the UV variable "outdoor work". (**A**) 690 men and 1221 women who have not worked/been outdoors for ≥4 hours daily during at least 25 years; (**B**) 298 men and 213 women who have worked/been outdoors for ≥4 hours daily during at least 25 years. In both outdoor groups (A and B), men have a higher wrinkle area than women in the lowest and middle age groups, only in the 65-75 years old group of B this is not statistically significant. In the highest age group (≥75 years) women have a higher wrinkle area than men, which is only statistically significant for group A.

A



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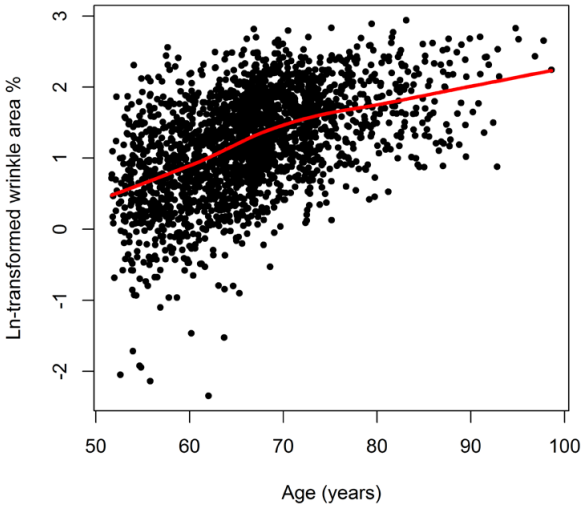

Supplementary Figure S2. Graphs of ln-transformed wrinkle area percentage by age, for men (**A**, N=1602) and women (**B**, N=2229) separately. The red line represents the regression line of the two variables.

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