Principal component analysis of seven skin aging features identifies three main types of skin aging

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ABSTRACT

Background: The underlying phenotypic correlations between wrinkles, pigmented spots (PS), telangiectasia and other related facial aging sub-phenotypes are not well understood.

Objectives: To analyze the underlying phenotypic correlation structure between seven features for facial aging: global wrinkling, perceived age (PA), Griffiths photodamage grading, PS, telangiectasia, actinic keratosis (AK) and keratinocyte cancer (KC).

Methods: This was a cross-sectional study. Facial photographs and a full-body skin examination were used. We used principal-component analysis (PCA) to derive principal components (PCs) of common variation between the features. We performed multivariable linear regressions between age, sex, body mass index, smoking, and UV-exposure and the PC scores derived from PCA. We also tested the association between the main PC scores and 140 single-nucleotide polymorphisms (SNPs) previously associated with skin aging phenotypes.

Results: We analyzed data from 1790 individuals with complete data on seven features of skin aging. Three main PCs explained 73% of the total variance of the aging phenotypes: a hypertrophic/wrinkling component (PC1: global wrinkling, PA and Griffiths grading), an atrophic/skin color component (PC2: PS and telangiectasia) and a cancerous component (PC3: AK and KC). The associations between lifestyle and host factors differed per PC. The strength of SNP associations also differed per component with the most SNP associations found with the atrophic component [e.g. the *IRF4* SNP (rs12203592); P-value = 1.84×10^{-22}].

Conclusions: Using a hypothesis-free approach, we identified three major underlying phenotypes associated with extrinsic aging. Associations between determinants for skin aging differed in magnitude and direction per component.

INTRODUCTION

Facial aging occurs alongside the progressive decline of functionality of all organs in the body that occurs over time. Facial aging has been partitioned into intrinsic and extrinsic features, each with overlapping and distinctive histological hallmarks[1]. Histologically, intrinsic aging is characterized by a loss of cells and extracellular matrix components from the dermal and epidermal layers[1]. Extrinsic aging is mostly due to chronic ultraviolet radiation (UVR) exposure and is characterized by deposits of abnormal amorphous proteins of abnormal elastin in the papillary dermis (solar elastosis) and inflammatory changes that lead to a thickened epidermis[2].

The alterations due to both intrinsic and extrinsic factors in the skin result in notable skin aging phenotypes (to varying degrees), namely: wrinkles (fine and coarse), pigmentation changes, telangiectasia and skin sagging. However, the latter is arguably mainly due to subcutaneous changes in the musculature and bone structure. When facial skin is assessed in the clinic, two main clinical types have been described: hypertrophic and atrophic aging[3]. Hypertrophic-type skin presents as leathery inelastic skin with coarse wrinkles and pigmentation changes; it mainly affects individuals with darker skin (i.e. Fitzpatrick skin type III). The atrophic variant is characterized by telangiectasia, hypertrophic sebaceous glands, and absence of wrinkles; it is more frequent in fair-skinned individuals[3]. In addition, people with the atrophic type have an increased risk for skin malignancies[4]. The above clinical definition assess mainly photoaging (damage due to UVR), although they also involve features of intrinsic skin aging such as fine wrinkling and atrophy[3]. From the above classification it is not clear how wrinkles, pigmented spots (PS), telangiectasia and keratinocyte growths are related to each other. Moreover, it is unclear which patients are at risk of these distinct phenotypes with respect to their exposure, lifestyle and genetics. In addition, the clinical assessment is difficult to quantify as it is based on the experience of the clinician doing the assessments.

Epidemiological studies have shown that in addition to UVR (sun exposure or tanning beds), smoking, body mass index (BMI)[5], hormones[5] and pollutants[6,7] are risk factors for facial aging. The role of genetic variation in skin aging is less well studied. Several genome-wide association studies (GWAS) have been published using different facial aging phenotypes[8,9], but only very few single-nucleotide polymorphisms (SNPs) have been identified and replicated to date. A possible explanation could be that the phenotypes used in these studies are too heterogeneous for the sample sizes of the GWAS used to date[10]. In addition, studies have rarely used the same phenotype to identify SNP associations; skin aging GWAS include perceived age (PA), wrinkles, loss of fine skin patterning[11] and scores of photoaging manually graded using facial photographs (e.g. Griffiths grading)[12]. However, it is not clear how these different phenotypes correlate to each other, making it difficult to compare results across studies.

For observational studies, where different determinants are investigated for associations with facial aging, it is important to have phenotypes that are relatively easy to measure in large series of individuals. Phenotypes derived from digital photographs are a good alternative because of

their objectivity and easy implementation for epidemiological and genetic studies of skin aging. As part of the dermatological investigations conducted in a population-based study (the Rotterdam Study)[13], fully standardized three-dimensional (3D) photographs of the face have been taken of participants to assess skin aging. So far, we have investigated the association between lifestyle factors and/or genetic variants to several skin aging-related features including PS[14], PA[15], global wrinkling[5], actinic keratosis (AK)[16] and keratinocyte cancer (KC)[17]. Interestingly, several determinants previously associated with skin aging showed contrasting effects across specific skin aging features (e.g. a pale skin color was associated with more PS but fewer wrinkles)[5]. We hypothesize that a better understanding of the phenotypic correlation structure between these different phenotypes will help to clarify such unexpected findings.

Here, we estimated the amount of shared variation between skin aging-related phenotypes (PA, Griffiths grading, AK and KC) using principal-component analysis (PCA)[18]. PCA can be used to investigate the underlying variance-covariance structure of multiple features across participants[18]. Using PCA we identified main components of common variation, and used these as dependent variables to test for the direction and magnitude of effects of both lifestyle factors and genetic variants previously associated with skin aging.

MATERIALS AND METHODS

Study population

The Rotterdam Study is an ongoing prospective population-based cohort study following 14,926 participants aged ≥ 45 years in Ommoord, a district of Rotterdam in the Netherlands, since 1990. Details of the study design and objectives have been described elsewhere[13]. The Rotterdam Study 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.

Facial skin aging features

Standardized high-resolution digital 3D facial photographs (Premier 3dMDface3-plus UHD, Atlanta, GA, U.S.A.) are being collected as part of dermatological examinations carried out in the Rotterdam Study. Between September 2010 and June 2014, a total of 4649 participants were photographed and examined at the research centre. Using frontal and 3D photographs, three main phenotypes were derived with semi-quantitative image analysis of frontal images, namely: PS, telangiectasia, and global wrinkling. A detailed description of PS and wrinkling is presented elsewhere[19]. In short, two-dimensional images were generated from the 3D photographs using Blender v.2.7 (http://www.blender.org). Next, the photographs were masked to isolate skin areas of interest using semi-automated masking (MATLAB, The MathWorks, Inc, Natick, MA, USA, ver-

sion 2013a)[19]. PS were digitally quantified as areas that are dark brown relative to the surrounding skin, with a roundish shape, present on the forehead, cheeks and nose[19]. Further, the percentage of skin area detected as hyperpigmented spots was calculated. Telangiectasia were quantified as areas that are colored red to purple and linear or branch-like in shape. Global wrinkling was quantified as a percentage of the masked facial skin area. These phenotypes have been validated against clinical assessment of the same photographs[19].

In addition, PA was included in the analysis because it has been used recently as a phenotype to investigate lifestyle factors and genes for skin appearance[8,15]. Independent observers who were blinded to chronological age assessed the same photos for PA using an ordinal scale of 10 age groups (with an average number of 27 assessments per image/participant and a minimum of 20)[15]. PA was calculated as the best linear unbiased predictor of the mean estimated age of the person in the image from a Proc-mixed model (SAS 9.3; SAS, Cary, NC, U.S.A.) with the viewing order as a fixed effect and assessor and image as random effects. For comparison, we also scored wrinkles by grading the facial photographs using the photonumerical grading scale from Griffiths *et al*[12]. We used nine ordinal levels from the 3D photos by four independent raters. Before phenotyping, a total of 100 photos (50% females) were randomly selected and openly discussed to reach a consensus between all raters. The concordance between the raters was evaluated using Pearson correlation coefficients and Cohen's kappa's coefficient. The average score of the four raters was considered as a quantitative phenotype.

Furthermore, the number of total AKs and KCs were also included. These were generated based on full-body examination.[20] AK was defined as rough red scaling lesions, not fitting another diagnosis and categorized into four levels, namely: 0, 1-3, 4-9 and ≥ 10.[16] KCs were assessed as a binary outcome [1: presence of KC: basal cell carcinoma (BCC) or squamous cell carcinoma (SCC); 0: absence]. In total, we analyzed seven features of skin aging.

Statistical analysis

Principal component analysis

Characteristics of the three main phenotypes (e.g. global wrinkles, PS and telangiectasia) and the associated variables (Griffiths grading, PA, AK and KC) are presented as means (SD) or medians (interquartile range) for non-normally distributed determinants and frequencies (percentages) for categorical determinants.

To investigate the underlying correlation structure of the seven features we used PCA as an exploratory analysis (details of the procedure and analysis to assess whether our data were suitable for PCA are presented in File S1, see Supporting Information). In short, PCA was carried out using the matrix correlations on complete cases and using varimax rotation, where residual correlation between latent components is not assumed. The loading factors (principal components, PCs) with eigenvalues higher than 1[21] were selected. The above analysis was performed using SPSS for Windows version 21.0 (SPSS; Chicago, IL, U.S.A.).

Multivariable linear regressions on demographic and lifestyle factors

Scores were derived from the main PCs and used as dependent variables to test for associations with main lifestyle factors, using multivariable linear regressions to calculate adjusted beta coefficients and 95% confidence intervals. Based on previous results from the Rotterdam Study[5], the following variables were tested: chronological age, sex, smoking status (never, former, current), skin color (pale, white and white-to-olive), BMI and four variables used as proxy to assess UVR exposure. Details on how the variables were defined are presented in File S1 (see Supporting Information).

Multivariable linear regressions on single-nucleotide polymorphisms involved in skin aging

To test for associations between previously identified genetic variants and PC scores we searched the literature for SNPs with genome-wide associations (P-value ≤ 5×10^{-8}) (Table S1, see Supporting Information) in at least two different studies with different skin aging phenotypes[14,15] and related phenotypes including AK[16], skin color[22], hair color[23] and eye color[14].

Next, we extracted the SNP allele scores (derived from imputation/genotypes) from an existing SNP GWAS dataset available from the Rotterdam Study [a description of how the genotypes were generated is presented in File S1 (see Supporting Information) and the references therein]. Further, we performed a linkage disequilibrium analysis to remove SNPs that were highly correlated with each other (see File S1, Fig. S1 and Table S2, Supporting Information). Lastly, we tested for per-SNP association with the three PCA-derived scores using a multivariable linear regression on complete cases. We adjusted this analysis for age, sex and four covariates used to correct for possible population stratification. The threshold for significance of the SNP associations was defined by dividing the nominal P-value by the total number of SNPs that show low correlations (P-value = 0.05/140; File S1, see Supporting Information). These analyses were performed using the R statistical package, v.5.1.3 (http://www.R-project.org).

RESULTS

Principal component analysis

In total, there were 1793 participants with complete data on the seven features. The mean distribution and standard error of the features we analyzed are presented in Table S3 (see Supporting Information). Table 1 presents Pearson's correlations derived from the PCA between global wrinkles, PS, telangiectasia and related features (PA, Griffiths grading, AK and KCs). Global wrinkling was poorly correlated with PS with a negative estimate of -0.20 and there was no correlation between global wrinkling and telangiectasia. Likewise, the correlation between PS and telangiectasia was only 0.27. On the other hand, strong correlations were found between global wrinkles and PA (0.65), and global wrinkles and Griffiths grading (0.74). Although AK has previ-

Table 1. Principal component analysis: pairwise correlations between seven facial skin phenotypes and skin cancer

	Global wrinkling	Pigmented spots	Telangiectasia	Perceived age	Griffiths grading	Actinic keratosis	Keratinocyte cancers
Global wrinkling	1.00						
Pigmented spots	**-0.20****	1.00					
Telangiectasia	**-0.24****	**0.27****	1.00				
Perceived age	**0.65****	**0.15****	0.01[a]	1.00			
Griffiths grading	**0.74****	**0.08****	**-0.06****	**0.75****	1.00		
Actinic keratosis	**0.15****	**0.04****	0.02[a]	**0.21****	**0.13****	1.00	
Keratinocyte cancers	**0.05****	**0.06***	-0.02[a]	**0.13****	**0.05****	**0.26****	1.00

*correlation is significant at the 0.05 level (one-tailed); **correlation is significant at the 0.01 level (one-tailed); [a]non-significant correlations.

ously been considered to be a marker of UVR-induced skin aging, it correlated poorly with the other skin features with correlations of 0.21 between AK and PA, and 0.26 between AK and KC.

Table 2 presents the main PC components derived from the PCA. The first three components had eigenvalues ≥ 1 and accounted for 73% of the variance. The first component (hypertrophic component; PC1) was strongly correlated with the wrinkling-related assessments (global wrinkles, Griffiths grading and PA) with correlations higher than 0.80 (Table 3). The second component (atrophic component; PC2) correlated with PS and telangiectasia, accounting for almost 82% of the variation of PS and 77% of telangiectasia. Global wrinkling was negatively correlated with PC2 (-0.32). AK and KC, on the other hand, were grouped together in a separate component (cancerous component; PC3), with correlations of 0.77 between AK and PC3 and 0.81 between KC and PC3.

Table 2. Principal component analysis. Total variance shown by components

	Initial eigenvalues		
Component	Total	% of variance	Cumulative %
1	2.52[a]	35.98	35.98
2	1.40[a]	20.04	56.02
3	1.16[a]	16.54	72.56
4	0.74	10.51	83.07
5	0.72	10.31	93.38
6	0.26	3.68	97.06
7	0.21	2.94	100.00

Kaiser–Meyer–Olkin measure of sampling adequacy: 0.663. [a]Three PCs account for 73% variation.

Table 3. Rotated components. Correlations between the seven phenotypes and the three main principal components

	Principal components		
	1	2	3
Global wrinkling	0.87	-0.32	0.05
Pigmented spots	0.07	0.82	0.05
Telangiectasia	-0.1	0.77	-0.02
Perceived age	0.89	0.15	0.15
Griffiths grading	0.93	0.04	0.02
Actinic keratosis	0.14	-0.004	0.77
Keratinocyte cancers	0	0.04	0.81

Multivariable regression analysis on lifestyle factors and single-nucleotide polymorphism variants

Using the PCA scores generated from the three main components (PC1, PC2 and PC3) as dependent variables, we tested for their association with a range of determinants (Table 4). Effect sizes and direction of effects were different for the determinants across the three PCs. For example, age had the largest effect on the wrinkling component (PC1). Light skin color was positively associated with PC2 and weakly associated with PC3, but negatively associated with PC1, although the latter was not significant. Current smoking was positively associated with PC1 and PC2 and negatively associated with PC3. BMI was negatively associated only with PC1, although the effect was rather small. Being female was positively associated with PC2 and PC3, and negatively with PC1. Of the four UVR-related variables available in the Rotterdam Study, sun-protective behavior was significantly associated with PC1 and PC3, and propensity to sunburn was positively associated with PC2 and PC3. Although the direction and effect of the associations cannot be directly extrapolated as these are statistical constructs, it does show that the associations differ per component.

We also conducted a candidate gene analysis on 140 SNPs from 75 genes and 51 intergenic regions (Table S4, see Supporting Information) with the three PCs. These SNPs have been associated previously with skin aging-related features in previous GWAS (see references in File S1, Supporting Information). A total of 17 SNPs showed significant association with at least one skin PC after multiple testing correction (Table S4, see Supporting Information). Some of these associations were stronger for the PCs than those from individual skin features (e.g. rs2233173, beside the *melan-A* gene, was significant for PC2 but only nominally significant for PS), although for other SNP associations the opposite was true (e.g. rs12203592; Table S5, see Supporting Information). There were stronger effect sizes for PC2 when compared with telangiectasia and PS, although this was not the case for all features (Fig. S2, see Supporting Information). Overall, PC2 showed the most significant associations (Fig. 1; Table S4, see Supporting Information), of which rs478882, a hair color-associated SNP, was the top hit (P-value = 3.52×10^{-14}). This SNP was also nominally significant with PC3. Other significant associations included the rs12203592 SNP (*IRF4* gene), which has previously been associated with skin color, AK and skin cancer. There were clusters of associations that were similar between PC2 and PC3 components, but not PC1, as well as associations that were specific to each PC (Fig. 1; Table S4, see Supporting Information). PC1, where global wrinkling, PA and Griffiths grading were clustered, had the weakest genetic associations. The most significant association found for this component was for rs12350739, an intergenic SNP that has been assigned to the *BNC2* gene in previous GWAS of skin color. The genes associated with the SNPs are listed in Table S4 (see Supporting Information).

Table 4. Statistical associations between main lifestyle risk factors and the scores derived from principal component analysis (PCA)

	PC1			PC2			PC3		
	Effect size (SE)	95% CI	P-value	Effect size (SE)	95% CI	P-value	Effect size (SE)	95% CI	P-value
Age	**0.08 (0.003)**	**[0.08 – 0.09]**	**$<2.0 \times 10^{-16}$**	0.001 (0.003)	[-0.004 – 0.01]	0.62	**0.04 (0.003)**	**[0.03 – 0.05]**	**$<2 \times 10^{-16}$**
Sex									
female	**-0.24 (0.04)**	**[-0.32 – -0.17]**	**7.1×10^{-10}**	**0.97 (0.04)**	**[0.89 - 1.05]**	**$<2.0 \times 10^{-16}$**	**0.24 (0.05)**	**[0.14 – 0.33]**	**4.7×10^{-7}**
male	ref	ref	ref	ref	ref	ref	ref	ref	ref
Skin color									
pale	-0.14 (0.09)	[-0.31 – 0.03]	0.10	**0.86 (0.09)**	**[0.69 – 1.04]**	**$<2.0 \times 10^{-16}$**	**0.22 (0.10)**	**[0.03 – 0.42]**	**2.7×10^{-2}**
white	-0.07 (0.06)	[-0.18 – 0.05]	0.24	**0.25 (0.06)**	**[0.13 – 0.37]**	**2.5×10^{-5}**	0.10 (0.07)	[-0.04 – 0.23]	0.15
white-to-olive	ref	ref	ref	ref	ref	ref	ref	ref	ref
BMI	**-0.03 (0.00)**	**[-0.04 – -0.02]**	**8.9×10^{-13}**	0.00 (0.00)	[-0.01 – 0.01]	0.89	0.00 (0.01)	[-0.01 – 0.01]	0.73
Smoking[a]									
current	**0.44 (0.06)**	**[0.33 – 0.56]**	**2.6×10^{-14}**	**0.22 (0.06)**	**[0.10 – 0.33]**	**2.9×10^{-4}**	**-0.16 (0.07)**	**[-0.30 – -0.03]**	**1.7×10^{-2}**
former	0.08 (0.04)	[-0.01 – 0.16]	9.4×10^{-2}	**0.13 (0.05)**	**[0.04 – 0.22]**	**4.0×10^{-3}**	-0.09 (0.05)	[-0.20 – 0.01]	7.8×10^{-2}
never	ref	ref	ref	ref	ref	ref	ref	ref	ref
Lived in a sunny country[b]	-0.04 (0.08)	[-0.20 – 0.12]	0.62	0.03 (0.08)	[-0.13 – 0.19]	0.73	0.15 (0.09)	[-0.034 – 0.34]	0.11
Sunburn easily[c]	-0.03 (0.04)	[-0.12 – 0.05]	0.45	**0.29 (0.04)**	**[0.20 – 0.37]**	**1.8×10^{-10}**	**0.22 (0.05)**	**[0.12 – 0.32]**	**2.4×10^{-5}**
Winter in a sunny country[d]	0.13 (0.09)	[-0.05 – 0.32]	0.16	-0.02 (0.10)	[-0.21 – 0.17]	0.81	0.17 (0.11)	[-0.05 – 0.39]	0.14
Sun-protective behavior[e]	**-0.09 (0.04)**	**[-0.17 – -0.01]**	**3.3×10^{-2}**	0.02 (0.04)	[-0.06 – 0.10]	0.66	**0.12 (0.05)**	**[0.020 – 0.21]**	**1.8×10^{-2}**

Abbreviations: BMI, body mass index; 95% CI, 95% confidence interval; PC, principal component; PC1, PC2 and PC3 refer to the PC scores computed from the PCA and were used here as dependent variables; SE, standard error. Boldface indicaties significant associations (P-value<0.05).

[a]cigars, cigarettes or pipe; [b]history of living in a sunny country > 1 year (reference group: no); [c]tendency to develop sunburn (reference group: low); [d]spending winter in sunny country (reference group: no or less than 1 month per year); [e]wearing sunglasses and/or a brimmed hat in the sunshine (reference group: never/almost never).

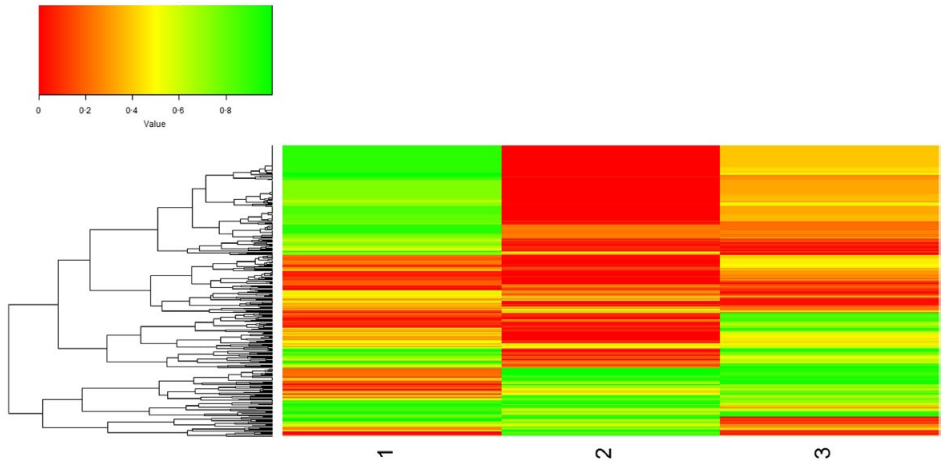

Figure 1. Heatmap of the correlation of associations between 140 single-nucleotide polymorphisms (SNPs) and the three principal components. The dendrogram on the left corresponds to clusters of SNPs with similar associations across the three components. The colors represent the strength of the associations (P-values) with red color for the most significant associations and green for the less significant associations.

DISCUSSION

The principal component analysis of seven skin features identified three main components of extrinsic skin aging that explained 73% of the total variance. Although it is difficult to give a biological label to these components, because these are statistical constructs, it could be argued that PC1 is related to hypertrophic skin aging[3], while PC2 corresponds to the atrophic component[3]. In contrast with earlier studies based on clinical assessments only, our analysis separated a cancerous component (PC3) from the other two PCs. This suggests that hypertrophic skin aging and atrophic skin aging occur via partly different pathophysiological mechanisms than those producing keratinocyte growths, in contrast to previous clinical-based studies[4]. The analysis presented is more robust compared with previous clinical classifications of photoaging as the latter are subjective and operator dependent and therefore more prone to bias, while our classification was based on a data-driven and hypothesis-free approach in a large population sample which is more likely to provide an objective differentiation between skin aging phenotypes.

The associations between the main lifestyle factors for skin aging and the PCs differed in magnitude and the direction of effects. Light skin color was positively associated with the atrophic component of skin aging (PC2) and weakly associated with the cancerous factor (PC3), but inversely associated with the hypertrophic factor (PC1), although it was not significant. This is more likely due to a reduced sample size because in our previous publication on the epidemiology of wrinkles, which included over 3000 participants, the association was significant[5]. Current smoking was positively associated with both hypertrophic and atrophic skin aging, but negatively associated with the cancer-related component (PC3). All these associations were significant (Table 4).

Although smoking is positively associated with SCC, the protective effect of smoking on KC has recently been shown for cutaneous BCC[24] and is in line with our results, as most of the KCs in our study were BCCs (80%).

The differential strength and direction of known determinants on PCs highlight the importance of understanding the different aspects of skin aging in general. This has clinical implications as, for example, individuals with a predominant hypertrophic component will benefit the most from interventions to modify lifestyle factors, while this may be less relevant for individuals with an atrophic component (except for UVR exposure, as these individuals will be more susceptible due to their genetic background as shown in Fig. 1). Furthermore, the fact that the cancerous component is separated from the atrophic one highlights the importance of screening for skin cancer even if individuals do not show strong signs of skin aging. The three components could help in the interpretation of results of other assessments of epidemiological studies on facial aging. For example, in a recent publication by Law *et al*.[11], the phenotype "microtopography scores" was used as an assessment for loss of fine skin patterning, also a feature of skin aging. In that article, SNPs associated with *IRF4* and *MC1R* genes, which are skin color genes, were significantly associated. The microtopography phenotype is in our view an atrophy-related phenotype, which explains the associations with skin color genes. Thus, in light of our analysis, it may be easier to understand results from other studies using our PCA-derived components than with individual's phenotypes.

To understand whether genetic correlations mirrored the phenotypic analysis, we calculated pairwise genetic correlations (the extent of shared variance between two traits due to genetic effects; see Table S6, Supporting Information) between the seven features and found higher correlation between phenotypes within the hypertrophic component, mostly driven by the correlations with wrinkling. The difference could be due to genetic correlations being less prone to residual confounding than the phenotypic ones. We also found a borderline significant negative correlation between PS and global wrinkling (-0.52; SE 0.27; P-value = 0.03; Table S6, see Supporting Information). The latter suggests that although some genetic variants may be associated with both traits, they could have opposite effects and, therefore, would cancel each other out if the phenotypes were combined together. However, our sample size is probably too modest for a robust estimation of genetic correlations and therefore larger samples may be needed to validate the findings. The per-SNP associations we found with atrophic skin aging (PC2) showed that the genetic variation of PS is largely determined by pigmentation genes, while these have very little influence on wrinkles, supporting our early GWAS findings on wrinkles.[9] Our study cautions against combining different features of skin aging into a single score without a previous understanding of the phenotype and genotypic correlations, as this can dilute the effects of individual genes and/or risk factors. Even combining moderately correlated phenotypes can decrease the power of genetic identification, as illustrated by the *IRF4* SNP (rs12203592), which had a weaker association with PC2 than for PS. This may be related to the fact that genetic associations are more susceptible to phenotypic heterogeneity than non-genetic association analysis and therefore other approaches for gene finding may be needed.

This study has several limitations. Firstly, the PCA is a statistical method that uses assumptions – such as there is little error in the measurement of the variables – that may not hold true in practice. In addition, we used a method (varimax approach) that assumes that there are no residual correlations between the main PCs. Residual correlations between the main PCs may still be significant due to, for example, shared determinants (e.g. UVR exposure, skin color). Although there are multivariable techniques that account for residual correlations between components, this would make the interpretation of the PCs more difficult. Furthermore, UVR-related variables used in this paper are more related to individual behavior towards sun exposure than actual UVR exposure. This limits the conclusions we can make for the association of these variables with the PCs. This issue is not limited to this study but is a known problem when measuring environmental exposure with questionnaire-based instruments in epidemiological research. This needs to be improved in the future. Lastly, although the PCA approach can be considered to be unbiased, the interpretation of what the PCs mean can be subject to debate. Here, we interpreted the PCs in the context of known literature on different clinical phenotypes and taking into account which original phenotypes were accounted for by the PCs. Because this is an exploratory PCA and not a confirmatory one, the interpretation of the PCA is largely research dependent. For example, it could be argued that PC2 is more a pigmentation component than an atrophic one because of the strong association of this component with pigmentation-related SNPs. We tested this by including skin color, eye color and hair color in the PCA and they formed a separate component (data not shown). However, with the added variables the new component explained less total phenotypic variance (four PCs, 66%; correlation between PS and PC4 was 44%) and did not improve the fitting of the data. Therefore, we did not include this component as a major skin aging component.

To conclude, using an unbiased approach in a large sample, we found three underlying phenotypes for photoaging, two of which matched the previously recognized clinical subtypes of photoaging, namely hypertrophic and atrophic skin aging. The cancerous component of skin aging adds to the complexity of the latter with a partial genetic overlap additionally found between PS and skin cancer, which can now be explored in further depth.

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