Journal of Dermatological Science 86 (2017) 21–29

Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com

Genetic variants associated with skin aging in the Chinese Han population

Wenshan Gao[a,b,1], Jingze Tan[c,1], Anke Hüls[d,1], Anan Ding[a], Yu Liu[a], Mary S. Matsui[f], Andrea Vierkötter[d], Jean Krutmann[d,2], Tamara Schikowski[d,***,2], Li Jin[a,c,e,**,2], Sijia Wang[a,c,*,2]

[a] Chinese Academy of Sciences Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] State Key Laboratory of Genetic Engineering and Ministry of Education Key Laboratory of Contemporary Anthropology, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai 200438, China
[d] IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
[e] Fudan-Taizhou Institute of Health Sciences, Taizhou, Jiangsu 225300, China
[f] The Estee Lauder Companies Inc, Melville, NY, United States, United States

ARTICLE INFO

Article history:
Received 19 August 2016
Received in revised form 30 November 2016
Accepted 21 December 2016

Keywords:
Skin aging
Chinese Han population
Candidate SNPs
Candidate genes

ABSTRACT

Background: The progression and manifestation of human skin aging has a strong genetic basis; however, most of the supporting evidence has been gathered in Caucasian populations. The genetic contribution to the variation in skin aging in non-Caucasian populations is poorly understood.
Objective: To investigate the genetic risk factors of relevance for skin aging in East Asians, we conducted the first candidate gene study for signs of skin aging in Han Chinese.
Methods: We collected skin aging and genotype data in 502 female Han Chinese from the Taizhou cohort. We evaluated skin aging by the validated skin aging score SCINEXA™. Confounding factors were assessed through a questionnaire. We obtained the genotype data for 21 candidate SNPs and for a further 509 SNPs from 16 related candidate genes. Associations were tested by linear and logistic regression analyses and adjusted for potential confounders.
Results: Our candidate study found a significant association between SNP rs2066853 in exon 10 of the aryl hydrocarbon receptor gene *AHR* and crow's feet. In addition, we found a significant association between SNP rs10733310 in intron 5 of *BNC2* and pigment spots on the arms, and between SNP rs11979919, 3 kb downstream of *COL1A2*, and laxity of eyelids.
Conclusions: Our results identified genetic risk factors for signs of skin aging (pigmentation, wrinkles or laxity) in Han Chinese. We also found that the manifestation of skin aging is further modified by anatomical site. Together with previous work, our results also suggest that different genetic variants could be responsible for distinct skin aging signs characteristic of Caucasians compared to East Asians.

Abbreviations: AhR, aryl hydrocarbon receptor; AM, arithmetic mean; AMR, arithmetic mean ratio; BMI, Body mass index; BNC2, basonuclin 2; CI, confidence interval; COL1A2, collagen type I alpha 2; GM, geometric mean; GMR, geometric mean ratio; SCINEXA™, Score for intrinsic and extrinsic skin aging; SNP, single nucleotide polymorphism.

* Corresponding author at: Shanghai Institutes of Biological Sciences, 320 Yue Yang Road, Shanghai 200031, China.
** Corresponding author at: School of Life Sciences, Fudan University, 220 Han Dan Road, Shanghai 200438, China.
*** Corresponding author at: IUF-Leibniz Research Institute for Environmental Medicine, Auf’m Hennekamp 50, 40225 Düsseldorf, Germany.
E-mail addresses: Tamara.Schikowski@IUF-Duesseldorf.de (T. Schikowski), lijin@fudan.edu.cn (L. Jin), wangsijia@picb.ac.cn (S. Wang).
[1] These authors contributed equally to this work.
[2] Co-senior authors.

http://dx.doi.org/10.1016/j.jdermsci.2016.12.017

1. Introduction

Skin aging has been categorized as extrinsic and intrinsic, reflecting the influence of environmental versus genetic factors in the variability of characteristic phenotypic markers [1,2]. Available data suggest that the manifestation of both intrinsic and extrinsic aging may have a strong genetic basis, which can make an individual more or less susceptible to specific skin aging signs [3].

Population based cohort studies assessing the effects of common single nucleotide polymorphisms (SNPs) have found that variants of the pigmentation genes *SLC45A2* in Asians [2] and *MC1R* (melanocortin 1 receptor) in Europeans [4] are associated with the presence of solar lentigines. Another candidate gene study by Elfakir et al. [5] has established an association between *MC1R* gene variants and an increased risk of photoaging. A recent genome-wide association study (GWAS) has linked facial photoaging to a SNP in the intronic area of the *STXBP5L* gene [6]. In addition, a Dutch study using data from the Rotterdam Cohort Study demonstrated that variants in the *IRF4, MC1R, ASIP*, and *BNC2* genes are significantly associated with Caucasian facial pigmentation spots [7], and that the *MC1R* gene is associated with perceived facial age [8]. However, most of these genetic variants associated with skin aging have been identified in Caucasians. The genetic basis for skin aging in non-Caucasian populations has remained poorly understood.

To the best of our knowledge, this is the first report of a candidate gene study conducted for skin aging signs in Han Chinese. We have tested previously reported candidate SNPs, as well as SNPs in related candidate genes, for association with signs of skin aging in a sample of Chinese women from the Taizhou cohort.

2. Materials and methods

2.1. Study population

Our study population included 502 female Han Chinese from Taizhou (China). These participants were recruited for a large prospective study, the Taizhou Longitudinal Study, which aims to investigate the genetic and environmental risk factors for common chronic diseases in China. The detailed characteristics of the Taizhou Longitudinal Study have been described elsewhere [9]. Between August and September 2012, we investigated 502 healthy Chinese women ranging in age from 32 to 85 years. Only individuals who had been residing for at least 15 years at their current address were included. The human ethics committee of Fudan University in Shanghai approved this study, and the principles of the Declaration of Helsinki were followed. All participants received detailed information on the study in writing and subsequently gave their written consent.

Table 2
Description of skin aging signs.

Skin aging signs	
Number of pigment spots:	
On forehead, GM (sd)	3.4 (2.3)
On cheeks, GM (sd)	4.9 (2.1)
On upper side of the forearm, GM (sd)	2.6 (2.4)
On back of the hand, GM(sd)	3.6 (2.2)
Score for coarse wrinkling:	
On forehead, AM(sd)	3.3 (1.5)
Frown lines, AM (sd)	2.7 (1.2)
In the crow's feet area, AM (sd)	2.4 (0.8)
Under the eyes, AM (sd)	2.4 (0.9)
On the upper lip, GM (sd)	2.7 (1.5)
Nasolabial fold, AM(sd)	3.3 (0.9)
Score for further skin aging symptoms:	
Laxity of eyelids,AM(sd)	2.6 (1.3)
Laxity of cheeks,GM(sd)	2.4 (1.4)

Abbreviations: AM (sd), arithmetic mean (standard deviation); GM (sd), geometric mean (standard deviation).

2.2. Assessment of skin aging symptoms and its influencing factors

Skin aging was clinically assessed by a validated skin aging scoring system, the SCINEXA™ (score of intrinsic and extrinsic skin aging) [10]. Extrinsic skin aging was represented by pigment spots

Table 1
Description of the study population.

Sample size		502
Age[years]	Mean(SD)	58(10)
	Min-Max	32–85
Body mass index (BMI) [kg/m2]	Mean(SD)	24.0 (3.1)
	Min-Max	16.6–35.4
Education		
Primary school or lower education	%Yes(n)	52.3(248)
Junior high school	%Yes(n)	34.0(161)
Senior high school	%Yes(n)	12.0(57)
Junior college or higher education	%Yes(n)	1.7(8)
Menopause	%Yes(n)	75.8(372)
Sun exposure	Mean(SD)	3.28(2.19)
Average hours outside per day under sun exposure after age of sixteen	Min-Max	0.4–12
Smoking		
Never smoked	%Yes (n)	95.8(474)
Ex-smokers	%Yes (n)	0.4 (2)
Current smokers	%Yes (n)	3.8 (19)
Passive smoking	%Yes (n)	48.8(233)
Type of fossil fuel used for cooking		
Electricity/gas only	%Yes(n)	54.8(275)
Both electricity/gas and coal/biomass	%Yes(n)	41.2(207)
Coal/biomass only	%Yes(n)	4.0(20)

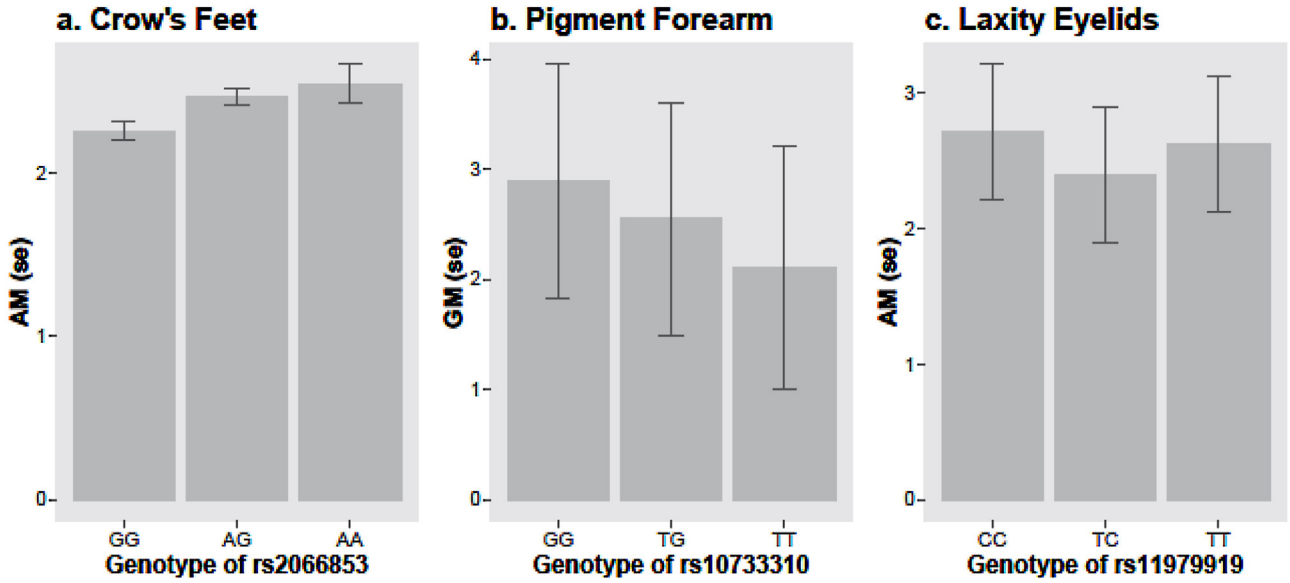

Fig 1. Description of skin aging signs as a function of the genotypes at associated SNPs. a, crow's feet; b, pigment forearm; c, laxity eyelids. Abbreviations: AM (sd), arithmetic mean (standard error); GM (sd), geometric mean (standard error).

(lentigines) and coarse wrinkles, whereas laxity was used to assess intrinsic skin aging. The number of pigment spots was evaluated as 0 = 0 pigment spots, 1 = 1–10 pigment spots, 2 = 11–50 pigment spots, and 3 = more than 50 pigment spots on forehead, cheeks, upper side of the forearms and back of the hands. Coarse wrinkles were assessed with scores ranging from 0 (absent) to 5 (severe) according to photo-reference scales on the forehead, between the eyebrows, in the crow's feet area, under the eyes, on the upper lip and the nasolabial fold. Laxity was assessed on eyelids and cheeks; it was also scored on a scale from 0 to 5.

Information about other known variables which might influence skin aging was collected through a standardized questionnaire-based interview. The questionnaire contained questions about age, body mass index (BMI), educational level (categorized as primary school, junior high school, senior high school and higher education), menopause (yes/no), sun exposure (average h outside per day in summertime after age of 16), smoking (never, ex and current smokers), passive smoke exposure (yes/no, at home and/or at work), and type of fossil fuel used for cooking (only use electricity/gas, both electricity/gas and coal/biomass, only use coal/biomass) [11].

2.3. Selection of candidate SNPs and candidate genes

We first compiled a list of 21 previously reported SNPs related to melanin synthesis, collagen synthesis, DNA repair and xenobiotic metabolism, and antioxidant systems, all of which could potentially cause skin aging (Supplementary Table 1). These 21 previously reported SNPs are referred to as "candidate SNPs"; they belong to 16 genes, which are referred to as "candidate genes".

Most of the 21 candidate SNPs were chosen from studies in Caucasian populations. However, different SNPs in or near the same genes may affect skin aging in East Asians. We therefore expanded our analysis to include 509 further SNPs in or near the same genes (for a description, see Supplementary Table 2). These SNPs are located within 5 kb (upstream or downstream) of each candidate gene.

The final set of SNPs used in this study comprised 530 SNPs (including the previously reported 21 SNPs) from 16 genes. The genotype data for all 530 SNPs were extracted from microarray data (Illumina Zhonghua v.1.1) that had been generated for a separate project. All 530 SNPs had a minor allele frequency > 1%, a call rate > 98%, and Hardy Weinberg P values < 0.05. Allele frequencies for all SNPs were taken from the Ensemble Genomes resource [12].`

2.4. Statistical analysis

To test genetic association, we applied generalized linear models, assuming an additive allele effect, where SNP genotypes were coded as 0–2, according to the number of minor alleles.

All regression models were adjusted for age, BMI (kg/m^2), smoking history, passive smoking, level of education, menopause, daily average sun exposure during adulthood, and indoor air pollution exposure (coal or biomass heating) [11]. A Bonferroni correction for multiple testing was applied to the raw P values. The adjusted regression coefficients were transformed to geometric mean ratios (GMR) for log-normally distributed symptoms with 95% CI, and for normally distributed symptoms to adjusted arithmetic mean ratios (AMR) with 95% CI. The formulas for GMR Eq. (1)) and AMR (Eq. (2)) are the following:

$$GMR_i = \exp(\beta_i) \quad (1)$$

$$AMR_i = \frac{\beta_i}{Mean} + 1 \quad (2)$$

Here, β_i represents the regression coefficient and "Mean" the mean score for a skin aging sign. The GMR and AMR are relative values for continuous variables and are comparable in their meaning to the OR. They are more easily interpreted than a simple regression coefficient. They describe the relative change in skin aging signs when the number of minor alleles at each SNP is increased by one. Like the OR, a GMR or AMR of 1 means that there is no association, a GMR or AMR < 1 means a negative association, and a GMR or AMR > 1 means a positive association.

We further used candidate-gene focused Manhattan plots, which we call "short Manhattan plots", to show the association

Table 3a
Association between candidate SNPs and pigmentation and laxity.

Function	Gene	SNP	Pigment Forehead		Pigment Cheeks		Pigment Forearm		Pigment Back Hands		Laxity Eyelids		Laxity Cheeks	
			P value	GMR[95% CI]	*P* value	GMR[95% CI]	*P* value	GMR[95% CI]	*P* value	GMR[95% CI]	*P* value	AMR[95% CI]	*P* value	GMR[95%CI]
Collagen Genes	ELN	rs2071307	0.69	1.03 [0.88;1.21]	0.07	1.15 [0.99;1.33]	0.87	1.01 [0.87;1.18]	0.39	1.07 [0.92;1.23]	0.11	0.95 [0.89;1.01]	0.32	1.03 [0.97;1.09]
	COL1A2	rs42524	0.59	1.06 [0.87;1.29]	0.61	1.05 [0.87;1.26]	0.63	1.05 [0.87;1.27]	0.15	1.14 [0.95;1.37]	0.98	1.00 [0.92;1.07]	0.95	1.00 [0.93;1.07]
DNA-Repair	XPC	rs2228001	0.60	0.97 [0.87;1.08]	0.19	1.07 [0.97;1.18]	0.72	1.02 [0.92;1.13]	0.45	0.96 [0.87;1.06]	0.92	1.00 [0.96;1.04]	0.05	0.96 [0.92;1.00]
	XRCC3	rs861539	0.68	1.06 [0.81;1.39]	0.10	1.24 [0.96;1.59]	0.87	1.02 [0.78;1.33]	0.06	1.27 [0.99;1.64]	0.54	1.03 [0.93;1.14]	0.53	1.03 [0.93;1.14]
Melanin Synthesis	BNC2	rs10756819	0.70	0.98 [0.89;1.08]	0.62	1.02 [0.93;1.13]	0.78	1.01 [0.92;1.12]	0.76	1.01 [0.92;1.11]	0.45	1.01 [0.98;1.05]	0.71	0.99 [0.96;1.03]
	DCT	rs1407995	0.23	0.93 [0.82;1.05]	0.89	1.01 [0.90;1.13]	0.35	1.06 [0.94;1.20]	0.84	0.99 [0.88;1.11]	0.39	1.02 [0.97;1.07]	0.51	1.02 [0.97;1.07]
	OCA2	rs7495174	0.44	0.96 [0.86;1.07]	0.81	1.01 [0.91;1.12]	0.85	0.99 [0.89;1.10]	0.72	0.98 [0.89;1.09]	0.43	0.98 [0.94;1.03]	0.69	1.01 [0.97;1.05]
	OCA2	rs1800414	0.90	1.01 [0.91;1.12]	0.12	0.93 [0.84;1.02]	0.07	0.91 [0.82;1.01]	**0.03**	**0.90 [0.82;0.99]**	0.09	0.97 [0.93;1.01]	0.21	0.98 [0.94;1.01]
	SLC24A5	rs1834640	0.61	0.96 [0.81;1.13]	0.39	0.93 [0.80;1.09]	0.48	0.94 [0.80;1.11]	0.50	0.95 [0.81;1.11]	0.19	1.04 [0.98;1.11]	0.96	1.00 [0.94;1.06]
	MC1R	rs2228479	0.58	1.04 [0.91;1.19]	0.48	0.96 [0.84;1.08]	0.98	1.00 [0.88;1.14]	0.97	1.00 [0.88;1.13]	0.31	1.03 [0.98;1.08]	**0.03**	**1.06 [1.01;1.11]**
	SLC45A2	rs26722	**0.01**	**1.15 [1.04;1.27]**	0.36	1.05 [0.95;1.15]	0.25	1.06 [0.96;1.17]	0.34	0.95 [0.87;1.05]	0.26	0.98 [0.94;1.02]	0.83	1.00 [0.96;1.03]
	ASIP	rs4911414	0.19	0.91 [0.78;1.05]	0.79	0.98 [0.86;1.13]	0.97	1.00 [0.87;1.16]	0.60	1.04 [0.91;1.19]	0.93	1.00 [0.94;1.05]	0.95	1.00 [0.94;1.05]
xenobiotic metabolism and antioxidant system	NAT2	rs1041983	0.53	1.03 [0.93;1.15]	0.33	1.05 [0.95;1.16]	0.25	0.94 [0.85;1.04]	0.53	1.03 [0.94;1.14]	0.51	0.99 [0.95;1.03]	0.12	1.03 [0.99;1.07]
	CYP1A1	rs1048943	0.08	0.89 [0.79;1.01]	0.45	0.96 [0.85;1.07]	0.56	1.04 [0.92;1.17]	0.77	0.98 [0.88;1.10]	0.66	1.01 [0.96;1.06]	0.22	1.03 [0.98;1.08]
	CYP1A1	rs4646421	0.11	0.91 [0.82;1.02]	0.94	1.00 [0.90;1.10]	0.58	1.03 [0.93;1.15]	0.13	0.92 [0.84;1.02]	0.85	1.00 [0.96;1.05]	**0.02**	**1.05 [1.01;1.09]**
	CYP1A1	rs4886605	0.08	1.09 [0.99;1.21]	0.47	1.03 [0.94;1.14]	0.94	1.00 [0.91;1.11]	**0.01**	**1.14 [1.04;1.25]**	0.54	0.99 [0.95;1.03]	**0.01**	**0.95 [0.92;0.99]**
	AHR	rs17779352	0.30	1.20 [0.85;1.71]	0.25	0.82 [0.59;1.15]	0.27	1.25 [0.85;1.84]	0.83	1.04 [0.75;1.44]	0.25	1.08 [0.94;1.21]	0.50	0.96 [0.84;1.09]
	AHR	rs2066853	0.81	1.01 [0.91;1.13]	0.81	1.01 [0.92;1.12]	0.47	1.04 [0.94;1.15]	0.33	1.05 [0.95;1.16]	0.11	1.03 [0.99;1.07]	0.92	1.00 [0.96;1.04]
	CYP1B1	rs2855658	0.89	0.99 [0.84;1.16]	0.18	0.90 [0.78;1.05]	0.41	1.07 [0.92;1.24]	0.28	1.09 [0.94;1.26]	0.73	1.01 [0.95;1.07]	0.27	1.03 [0.97;1.10]
	CYP1B1	rs1056836	0.94	0.99 [0.85;1.16]	0.22	0.91 [0.79;1.06]	0.62	1.04 [0.89;1.21]	0.29	1.08 [0.94;1.25]	0.85	1.01 [0.95;1.06]	0.40	1.03 [0.97;1.09]
	SOD2	rs4880	0.48	0.95 [0.81;1.10]	0.34	0.93 [0.81;1.08]	**0.04**	**0.85 [0.74;0.99]**	0.41	0.94 [0.82;1.09]	0.57	0.98 [0.93;1.04]	0.95	1.00 [0.94;1.06]

Abbreviations: GMR, geometric mean ratio; AMR, arithmetic mean ratio; 95% CI, 95% confidence interval.
P values were adjusted for multiple testing by Bonferroni correction. Here, we corrected for the number of SNPs (N = 21) used in the analysis.
Suggestive associations with a raw P-value < 0.05 are printed in bold.

between all 530 SNPs and each skin aging sign. The X-axis of these plots shows the positions of SNPs within candidate genes. The Y-axis shows the observed -log10 p-values for the association.

3. Results

3.1. Characteristics of study populations

We investigated skin aging signs in 502 Han Chinese women of the Taizhou study cohort. The mean age of the sample was 58 (±10) years. The mean body mass index (BMI) was 24 kg/m^2. More than 50% of the women had an educational level lower than junior high school and used only clean fuels, including electricity and gas, for cooking. The majority of women were in menopause. Most women had never been smokers (95.8%), but half of them had passive smoke exposure (48.8%). See Table 1 for a description of relevant information for the study population.

3.2. Clinical skin aging manifestation

Skin aging traits were assessed by SCINEXA™ (score of intrinsic and extrinsic skin aging). The mean scores for the different skin aging signs are presented in Table 2. The geometric mean of score values of pigment spots ranged from 2.6 on the upper side of forearm to 5.0 on the cheeks. The mean score for all wrinkles at different locations of the face was around 3.0. The mean grade of laxity on eyelids and cheeks was about 2.5.

3.3. Association between candidate SNPs and occurrence of skin aging signs

First, we tested the association between the twelve skin aging traits and 21 previously reported candidate SNPs (description see Supplementary Table 1). We found that rs2066853 in exon 10 of *AHR* (aryl hydrocarbon receptor) was significantly associated with crow's feet, even after correcting for multiple testing (7%Δ per

Table 3b
Association between candidate SNPs and wrinkle formation.

Function	Gene	SNP	Wrinkles Forehead		Frown Lines		Crow's Feet		Wrinkles Under Eyes		Wrinkles Upper Lip		Nasolabial Fold	
			P value	AMR[95% CI]	*P* value	AMR[95% CI]	*P* value	AMR[95% CI]	*P* value	AMR[95% CI]	*P* value	GMR[95% CI]	*P* value	AMR[95%CI]
Collagen Genes	ELN	rs2071307	0.58	0.98 [0.91;1.05]	0.13	0.95 [0.88;1.02]	**0.03**	**0.93 [0.87;0.99]**	0.56	0.98 [0.92;1.04]	0.82	0.99 [0.95;1.04]	0.11	0.97 [0.93;1.01]
	COL1A2	rs42524	0.26	0.95 [0.86;1.04]	0.69	0.98 [0.90;1.07]	0.95	1.00 [0.93;1.08]	0.05	1.07 [1.00;1.15]	0.27	1.03 [0.98;1.09]	0.88	1.00 [0.95;1.05]
DNA-Repair	XPC	rs2228001	0.95	1.00 [0.95;1.05]	0.78	1.01 [0.96;1.06]	0.10	0.96 [0.92;1.01]	0.19	0.97 [0.93;1.01]	0.48	1.01 [0.98;1.04]	0.23	0.98 [0.95;1.01]
	XRCC3	rs861539	0.87	0.99 [0.86;1.11]	0.13	1.10 [0.97;1.22]	0.45	1.04 [0.93;1.15]	0.66	1.02 [0.92;1.13]	0.32	1.04 [0.96;1.13]	0.62	0.98 [0.91;1.05]
Melanin Synthesis	BNC2	rs10756819	0.84	1.00 [0.95;1.04]	0.07	0.96 [0.91;1.00]	0.34	1.02 [0.98;1.06]	0.75	0.99 [0.95;1.03]	0.89	1.00 [0.97;1.03]	0.95	1.00 [0.97;1.03]
	DCT	rs1407995	0.18	1.04 [0.98;1.10]	0.69	1.01 [0.96;1.07]	**0.02**	**1.06 [1.01;1.11]**	0.25	1.03 [0.98;1.08]	0.73	0.99 [0.96;1.03]	0.37	0.99 [0.95;1.02]
	OCA2	rs7495174	0.88	1.00 [0.95;1.05]	0.48	1.02 [0.97;1.07]	0.52	1.01 [0.97;1.06]	0.35	1.02 [0.98;1.06]	0.25	0.98 [0.95;1.01]	0.85	1.00 [0.97;1.03]
	OCA2	rs1800414	0.69	0.99 [0.94;1.04]	0.41	0.98 [0.93;1.03]	0.56	0.99 [0.95;1.03]	0.78	1.01 [0.97;1.05]	0.76	1.00 [0.97;1.04]	0.45	0.99 [0.96;1.02]
	SLC24A5	rs1834640	0.15	0.94 [0.87;1.02]	0.56	0.98 [0.90;1.05]	0.54	1.02 [0.95;1.09]	0.36	1.03 [0.97;1.10]	0.79	0.99 [0.95;1.04]	0.25	0.97 [0.93;1.02]
	MC1R	rs2228479	0.30	1.03 [0.97;1.09]	0.50	0.98 [0.92;1.04]	0.33	1.03 [0.97;1.08]	0.09	1.04 [0.99;1.10]	0.64	0.99 [0.95;1.03]	0.39	1.02 [0.98;1.05]
	SLC45A2	rs26722	0.76	0.99 [0.95;1.04]	0.38	1.02 [0.97;1.07]	0.89	100 [0.96;1.04]	0.87	1.00 [0.96;1.04]	0.70	1.01 [0.98;1.04]	0.85	1.00 [0.97;1.02]
	ASIP	rs4911414	0.57	1.02 [0.95;1.09]	0.40	0.97 [0.91;1.04]	0.37	0.97 [0.92;1.03]	0.79	0.99 [0.93;1.05]	0.77	0.99 [0.95;1.04]	0.72	0.99 [0.95;1.03]
xenobiotic metabolism and antioxidant system	NAT2	rs1041983	0.90	1.00 [0.95;1.04]	0.61	1.01 [0.97;1.06]	0.70	1.01 [0.97;1.05]	0.17	0.97 [0.93;1.01]	0.31	1.02 [0.99;1.05]	**0.01**	**1.03 [1.01;1.06]**
	CYP1A1	rs1048943	0.10	0.95 [0.90;1.01]	0.38	0.97 [0.92;1.03]	0.80	0.99 [0.95;1.04]	0.82	0.99 [0.95;1.04]	0.36	1.02 [0.98;1.05]	0.94	1.00 [0.97;1.03]
	CYP1A1	rs4646421	0.73	0.99 [0.94;1.04]	0.53	0.98 [0.94;1.03]	0.74	1.01 [0.96;1.05]	0.83	1.00 [0.95;1.04]	0.13	1.02 [0.99;1.06]	0.85	1.00 [0.97;1.03]
	CYP1A1	rs4886605	0.71	1.01 [0.96;1.05]	0.39	1.02 [0.97;1.06]	0.88	1.00 [0.96;1.04]	0.40	0.98 [0.94;1.02]	0.09	0.98 [0.95;1.00]	0.65	0.99 [0.97;1.02]
	AHR	rs17779352	0.31	0.92 [0.76;1.08]	0.86	1.01 [0.86;1.17]	**0.01**	**0.82 [0.68;0.96]**	0.89	1.01 [0.87;1.15]	0.73	1.02 [0.92;1.13]	0.20	0.94 [0.85;1.03]
	AHR	rs2066853	0.34	1.02 [0.97;1.07]	0.73	0.99 [0.94;1.04]	**2.1E-3**	**1.07 [1.02;1.11]**	0.38	1.02 [0.98;1.06]	0.74	0.99 [0.96;1.03]	0.74	1.00 [0.97;1.02]
	CYP1B1	rs2855658	0.70	0.99 [0.91;1.06]	0.14	1.05 [0.98;1.12]	0.42	1.03 [0.96;1.09]	0.31	0.97 [0.91;1.03]	0.24	1.03 [0.98;1.08]	0.65	0.99 [0.95;1.03]
	CYP1B1	rs1056836	0.66	0.98 [0.91;1.05]	0.23	1.04 [0.97;1.11]	0.74	1.02 [0.96;1.08]	0.30	0.97 [0.91;1.03]	0.23	1.03 [0.98;1.08]	0.57	0.99 [0.95;1.03]
	SOD2	rs4880	0.90	1.00 [0.93;1.06]	0.21	1.04 [0.98;1.11]	0.89	1.00 [0.94;1.06]	0.46	0.98 [0.92;1.04]	0.91	1.00 [0.96;1.05]	0.81	1.00 [0.96;1.03]

Abbreviations: GMR, geometric mean ratio; AMR, arithmetic mean ratio; 95% CI, 95% confidence interval.
P values were adjusted for multiple testing by Bonferroni correction. Here, we corrected for the number of SNPs (N = 21) used in the analysis.
Suggestive associations with a raw P-value < 0.05 are printed in bold; significant associations with an adjusted *P* value < 0.05 (raw *P* value < 0.0023 are underlined.

allele, $P = 0.002$). The minor allele A of rs2066853 showed an additive effect, which increased the grade of crow's feet by 7% per copy (Fig. 1a, Table 3b). Allele frequencies at rs2066853 differ between East Asians (CHB from 1000 Genomes Project Phase 3) and Caucasians (CEU from 1000 Genomes Project Phase 3). The ancestral allele A has a frequency of 40.2% in East Asians, but only of 11.5% in Caucasians (Supplementary Table 3). No other significant association was found (Table 3a, Table 3b).

3.4. Association between candidate genes and occurrence of skin aging signs

Most of the 21 candidate SNPs were chosen from studies in Caucasian populations. However, different SNPs in or near the same genes may affect skin aging in other populations (e.g. East Asians). We therefore expanded our analysis to 509 additional SNPs in or near the same genes (for a full description, see Supplementary Table 2).

In this candidate gene analysis, we found that rs10733310 in intron5 of the basonuclin 2 gene, *BNC2*, was significantly associated with pigment spots on the arms (−19%Δ per allele, $P = 7.9 \times 10^{-5}$) (Fig. 1b, Table 4a, Fig. 2b).The minor allele, T, at rs10733310 showed an additive protective effect on the number of pigment spots on the forearm. The geometric mean score for pigment spots on the forearm decreased by 19% for each T allele at rs10733310.

Furthermore, the SNP rs11979919, located 3 kb downstream of the collagen type I alpha 2 gene, *COL1A2*, was found to be significantly associated with laxity of eyelids (−26%Δ per allele, $P = 9.5 \times 10^{-5}$) (Fig. 1c, Table 4a, Fig. 2c). The minor allele, T, at rs11979919 showed an additive protective effect on the grade of eyelid laxity: the grade of laxity of eyelids decreased by 26% for each copy of this allele. Allele frequencies at these two SNPs vary between East Asians (CHB from 1000 Genomes Project Phase 3) and Caucasians (CEU from 1000 Genomes Project Phase 3) (Supplementary Table 3). Furthermore, a marginal signal was

Table 4a
Association between candidate genes and pigmentation and laxity.

Function	Gene	SNP	Pigment Forehead		Pigment Cheeks		Pigment Forearm		Pigment Back Hands		Laxity Eyelids		Laxity Cheeks	
		Number	*P* value	GMR[95% CI]	*P* value	GMR[95% CI]	*P* value	GMR[95% CI]	*P* value	GMR[95% CI]	*P* value	AMR[95% CI]	*P* value	GMR[95%CI]
Melanin synthesis	ASIP	3	0.23	0.94 [0.83;1.04]	0.15	0.93 [0.83;1.03]	0.07	1.11 [1.00;1.22]	0.70	0.98 [0.88;1.08]	0.44	1.04 [0.94;1.15]	0.25	1.02 [0.98;1.06]
	BNC2	180	0.01	1.17 [1.05;1.30]	0.04	1.19 [1.03;1.35]	**7.9E-5**	**0.81 [0.71;0.91]**	0.01	0.83 [0.70;0.96]	0.01	1.15 [1.05;1.26]	0.01	1.09 [1.02;1.15]
	DCT	12	0.17	0.87 [0.67;1.07]	0.31	1.07 [0.94;1.21]	0.05	1.12 [1.01;1.24]	0.22	0.89 [0.71;1.08]	0.17	1.11 [0.96;1.25]	0.27	1.03 [0.98;1.08]
	MC1R	11	0.28	1.08 [0.94;1.22]	0.23	1.09 [0.95;1.23]	0.52	0.88 [0.47;1.28]	0.13	0.74 [0.36;1.13]	0.08	1.44 [1.03;1.86]	0.50	0.99 [0.95;1.02]
	OCA2	138	0.02	0.87 [0.75;0.99]	0.01	0.85 [0.73;0.98]	0.01	0.85 [0.73;0.98]	0.01	1.67 [1.31;2.03]	0.01	0.84 [0.72;0.97]	0.01	0.94 [0.90;0.98]
	SLC24A5	4	0.08	1.09 [0.99;1.19]	0.05	0.91 [0.81;1.00]	0.24	1.06 [0.96;1.16]	0.18	0.92 [0.81;1.04]	0.33	1.09 [0.92;1.26]	0.38	0.98 [0.95;1.02]
	SLC45A2	29	0.01	0.87 [0.76;0.98]	0.05	1.26 [1.03;1.50]	0.06	0.76 [0.48;1.05]	0.05	1.11 [1.01;1.21]	0.07	1.13 [1.00;1.26]	0.08	0.91 [0.82;1.01]
Collagen genes	COL1A2	28	0.14	0.89 [0.73;1.05]	0.12	1.08 [0.98;1.18]	0.00	0.73 [0.53;0.94]	0.07	0.91 [0.81;1.01]	**9.5E-5**	**0.74 [0.60;0.89]**	0.02	0.96 [0.92;0.99]
	ELN	11	0.16	1.25 [0.94;1.56]	4.4E-3	0.85 [0.73;0.96]	0.05	1.11 [1.00;1.21]	0.02	0.79 [0.60;0.99]	0.20	0.87 [0.67;1.08]	0.02	1.05 [1.01;1.08]
Xenobiotic metabolism and antioxidant system	AHR	15	0.18	1.20 [0.93;1.46]	0.29	1.15 [0.89;1.41]	0.12	0.91 [0.79;1.03]	0.07	0.90 [0.79;1.01]	0.05	1.11 [1.01;1.22]	0.07	1.09 [0.99;1.18]
	CYP1A1	2	0.26	1.08 [0.95;1.21]	0.60	0.97 [0.84;1.09]	0.40	0.91 [0.69;1.13]	0.21	1.15 [0.93;1.36]	0.88	1.02 [0.79;1.25]	0.29	0.96 [0.88;1.03]
	CYP1B1	15	0.12	0.84 [0.62;1.06]	0.04	1.24 [1.03;1.45]	0.11	1.12 [0.98;1.25]	0.23	1.11 [0.94;1.28]	0.24	1.14 [0.92;1.36]	0.02	0.95 [0.90;0.99]
	NAT2	33	0.18	0.83 [0.57;1.10]	0.08	1.19 [0.99;1.38]	0.15	0.86 [0.66;1.06]	0.02	1.16 [1.04;1.29]	0.04	0.88 [0.76;1.00]	4.2E-3	1.07 [1.02;1.11]
	SOD2	8	0.04	0.79 [0.56;1.02]	0.32	0.95 [0.85;1.05]	0.01	0.73 [0.51;0.96]	0.33	0.90 [0.68;1.11]	0.10	0.92 [0.81;1.02]	0.08	0.97 [0.93;1.00]
DNA repair	XPC	10	0.18	1.08 [0.97;1.20]	0.14	0.92 [0.81;1.03]	0.16	1.19 [0.95;1.44]	0.06	1.11 [1.00;1.22]	0.15	1.20 [0.95;1.44]	0.04	0.92 [0.83;1.00]
	XRCC3	10	0.44	1.09 [0.87;1.31]	0.02	1.14 [1.03;1.24]	0.12	0.92 [0.81;1.03]	0.02	1.27 [1.06;1.48]	0.04	0.89 [0.77;1.00]	0.06	0.96 [0.93;1.00]

We used the lowest *P* value obtained for SNPs in one gene to show the significance of the association between a candidate gene and skin aging trait. *P* values were adjusted for multiple testing by Bonferroni correction. Here, we corrected for the number of SNPs (N = 509) used in the analysis.
Significant associations with an adjusted *P* value < 0.05 (raw *P* value < 9.8E-5) are printed in bold and underlined.
SNP Number: number of included SNPs per gene.

found for the association between wrinkles on the upper lip and *BNC2* with a minimum *P* value of 3.1×10^{-4} of rs7025750 (Table 4b).

4. Discussion

We performed an association study of candidate SNPs and candidate genes for signs of skin aging in 502 female Han Chinese from the Taizhou cohort. Our candidate SNP study showed that rs2066853 in exon 10 of the *AHR* gene was significantly associated with crow's feet. Furthermore, our candidate gene study identified an association between rs10733310 in intron 5 of *BNC2* and pigment spots on arm, and between rs11979919, located 3 kb downstream of *COL1A2*, and eyelid laxity.

Rs2066853 is the best studied SNP in the *AHR* gene to date. Its functional relevance for, and genetic association with, different phenotypes is well established. Nevertheless, to the best of our knowledge, we are the first to show an association with skin aging. Rs2066853 is located in exon 10 of the *AHR* gene, a region associated with transactivation of other genes [13], and leads to an amino acid change (Arg554Lys). In a study of coke-oven workers by Chen et al. (2006), a higher level of DNA damage was observed in participants carrying one or two A alleles at rs2066853, than in GG homozygotes [14]. Furthermore, GA/AA genotypes have been associated with a higher risk of colorectal polyp development after meat consumption compared to the GG genotype [15]. Finally, this SNP has been associated with a higher susceptibility to the effect of environmental exposures to substances such as PAH and dioxin-like chemicals [16,17]. This is congruent with our findings because crow's feet are part of extrinsic skin aging, and therefore a mainly environmentally induced sign of skin aging.

Our candidate gene study identified an association between rs10733310 inintron 5 of *BNC2*, and pigment spots on the arms. This is in line with the results of previous studies showing that variants in *BNC2* are associated with skin color [18], skin freckling [19] and the development of facial pigment spots [7]. However, all prior studies were performed in Caucasians. Ours is therefore the first study to show an association between variants of *BNC2* and pigmentation in Chinese.

In addition, we found rs11979919, located 3 kb downstream of *COL1A2*, to be associated with eyelid laxity. Splice site mutations in the *COL1A2* gene of type I collagen can give rise to forms of Ehlers-Danlos syndrome (EDS) due to the partial or complete skipping of exon 6, as well as to mild, moderate, or lethal forms of osteogenesis imperfecta as a consequence of the skipping of other exons [20–22]. Ehlers-Danlos syndrome includes skin hyperextensibility and sagging eyelids caused by genetic defects in collagens I and V [23], which is consistent with our findings.

Therefore, the SNPs rs10733310 (*BNC2*, associated with pigment spots on the arm) and rs11979919 (*COL1A2*, associated with eyelid

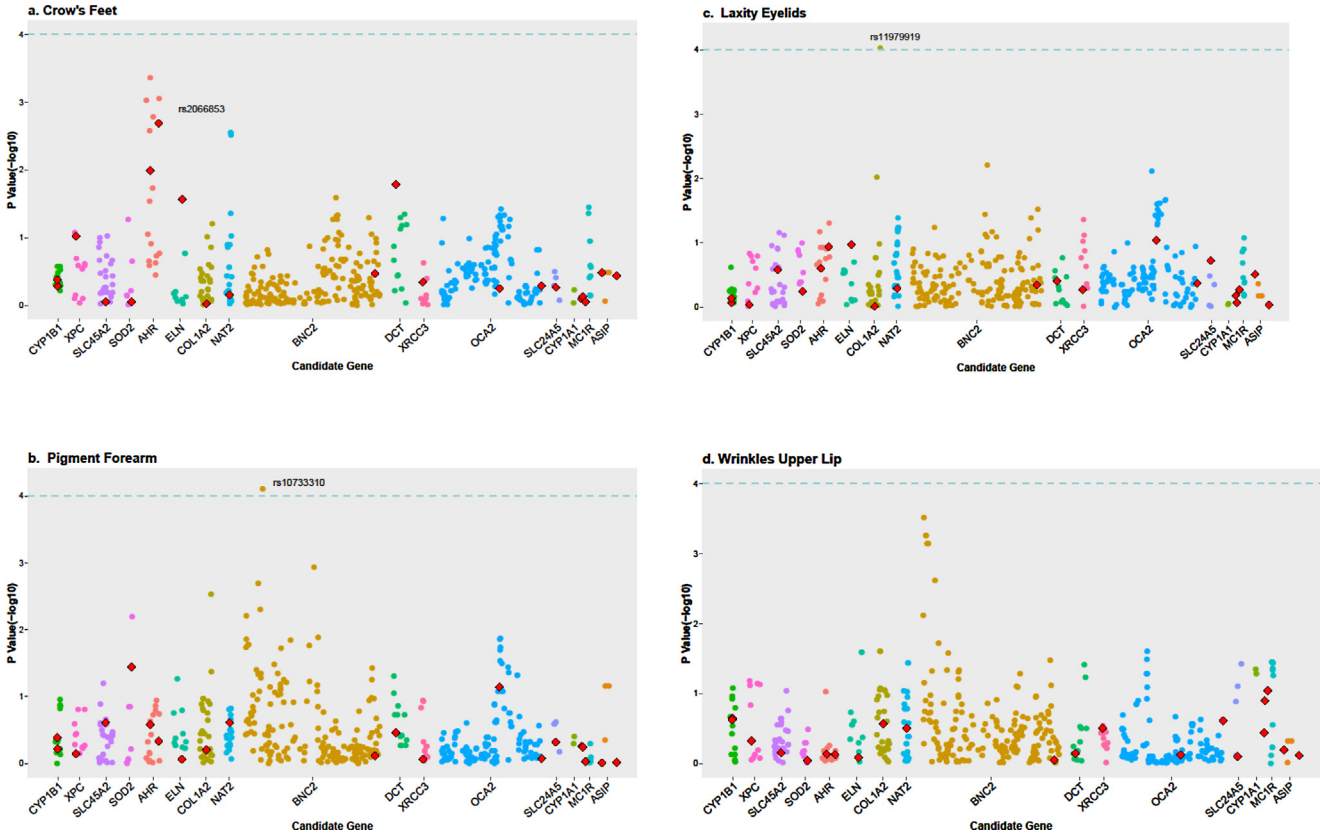

Fig. 2. Short Manhattan plots of the significant and marginal association.
The Y-axis shows -log10 p-values for the association between each SNP and skin aging. The X-axis shows the position of all 530 SNPs along the candidate genes. The 21 original candidate SNPs are represented by diamonds, whereas the 509 additional candidate gene SNPs are represented by dots. The horizontal line indicates the significance threshold of $P = 9.8 \times 10^{-5}$. *P* values were adjusted for multiple testing by Bonferroni correction. Here, we corrected for the number of SNPs (N = 509) used in the candidate gene analysis.

laxity) are both located in genes, for which an association with skin-related phenotypes has been previously reported. However, the SNPs themselves have never previously been reported in this context in the literature. One reason for this might be that most studies to date were performed in Caucasians, in whom different genetic variants could be responsible for skin aging compared to East Asian populations. More studies in East Asians are needed to validate these findings. Furthermore, it is worth noting that the SNPs found here to be associated with skin aging may not be the causal SNPs responsible for the phenotype variation, but rather a genetic marker in linkage disequilibrium with an as yet unidentified causal SNP or other sequence or structural variant in the genome. Further studies are required to pinpoint these causal variants.

Limitations of our study include the relatively modest sample size (n = 502) and the restriction to female subjects. To increase the power to detect associations despite these limitations, we focused on a candidate gene approach. A particular strength of our study is the use of the SCINEXA™ system for the evaluation of skin aging: it enables us to distinguish between the type and the anatomical site of skin aging and thereby results in a standardized measurement of skin aging, which allows for comparisons between studies.

In conclusion, our candidate gene study identified genetic risk factors for the signs of skin aging (pigmentation, wrinkles or laxity), which are further modified by anatomical site, in a Han Chinese population. We showed that a genetic variant in the *AHR* gene was significantly associated with the presence of crow's feet, variants in the *BNC2* gene, with pigment spots on the arms, and variants in *COL1A2*, with laxity of eyelids. Additional, larger cohort studies should be pursued in order to further clarify the genetic risk factors underlying each skin aging sign, as well as their interaction with environmental factors.

Funding

This work was funded by a Max Planck-CAS Paul Gerson Unna Independent Research Group Leadership Award (to S.W.), a National Thousand Young Talents Award from the Organization Department of the Central Committee of the CPC (to S.W.), an Excellent Young Scientists Fund Award from the National Science Foundation of China, a Chinese Academy of Sciences Visiting Professor Fellowship (PIFI; to J.K.), and a grant from the 111 Project (B13016) of Fudan University (to J.K.). This study is part of an International Leibniz Research Project between IUF and PICB/Fudan University. Finally, the study was financially supported by research grants from 'The Estee Lauder Companies'.

Conflict of interest disclosures

Mary Matsui is an employee of 'The Estee Lauder Companies'.

Table 4b
Association between candidate genes and wrinkle formation.

Function	Gene	SNP	Wrinkles Forehead		Frown Lines		Crow's Feet		Wrinkles Under Eyes		Wrinkles Upper Lip		Nasolabial Fold	
		Number	*P* value	AMR[95% CI]	*P* value	AMR[95% CI]	*P* value	AMR[95% CI]	*P* value	AMR[95% CI]	*P* value	GMR[95% CI]	*P* value	AMR[95%CI]
Melanin synthesis	ASIP	3	0.01	0.94 [0.89;0.99]	0.04	1.05 [1.00;1.11]	0.32	1.02 [0.98;1.07]	0.30	1.02 [0.98;1.07]	0.48	1.02 [0.97;1.06]	0.34	1.01 [0.99;1.04]
	BNC2	180	2.5E-3	1.08 [1.03;1.13]	0.02	1.06 [1.01;1.11]	0.03	0.95 [0.91;0.99]	1.4E-3	1.07 [1.03;1.11]	3.1E-4	1.11 [1.05;1.16]	0.01	1.13 [1.03;1.23]
	DCT	12	3.9E-3	1.15 [1.05;1.25]	0.20	1.04 [0.98;1.11]	0.05	1.09 [1.00;1.18]	0.02	1.10 [1.02;1.19]	0.04	1.09 [1.01;1.17]	0.09	0.97 [0.93;1.01]
	MC1R	11	0.03	0.93 [0.87;0.99]	0.09	1.16 [0.98;1.35]	0.04	0.94 [0.89;1.00]	0.10	0.96 [0.90;1.01]	0.04	1.04 [1.00;1.09]	0.04	1.11 [1.01;1.22]
	OCA2	138	0.03	0.80 [0.62;0.98]	0.06	0.83 [0.66;1.00]	0.04	0.94 [0.89;1.00]	0.05	1.05 [1.00;1.09]	0.03	1.12 [1.02;1.22]	0.01	1.12 [1.03;1.22]
	SLC24A5	4	0.05	0.95 [0.91;1.00]	0.39	0.98 [0.93;1.03]	0.32	1.03 [0.97;1.10]	4.8E-3	0.94 [0.90;0.98]	0.04	1.05 [1.00;1.10]	0.45	1.01 [0.98;1.04]
	SLC45A2	29	0.14	1.04 [0.99;1.10]	0.19	1.08 [0.96;1.19]	0.09	1.04 [0.99;1.09]	0.01	1.07 [1.02;1.11]	0.09	1.04 [0.99;1.09]	0.08	1.03 [1.00;1.06]
Collagen genes	COL1A2	28	0.04	1.08 [1.01;1.15]	0.03	1.08 [1.01;1.15]	0.06	0.95 [0.89;1.00]	0.03	0.93 [0.88;0.99]	0.03	1.05 [1.01;1.09]	0.01	0.94 [0.89;0.98]
	ELN	11	0.24	1.04 [0.97;1.1]	0.34	1.02 [0.97;1.07]	0.17	0.94 [0.86;1.02]	0.01	0.88 [0.78;0.97]	0.03	0.92 [0.84;0.99]	0.43	0.98 [0.92;1.03]
Xenobiotic metabolism and antioxidant system	AHR	15	0.12	1.04 [0.99;1.10]	0.25	1.03 [0.98;1.07]	4.4E-4	1.08 [1.03;1.12]	0.10	1.09 [0.98;1.19]	0.10	1.09 [0.99;1.19]	0.07	1.12 [0.99;1.26]
	CYP1A1	2	0.50	0.96 [0.86;1.07]	0.15	1.07 [0.97;1.18]	0.58	0.98 [0.89;1.06]	0.13	0.93 [0.84;1.02]	0.05	0.95 [0.90;1.00]	0.17	0.96 [0.90;1.02]
	CYP1B1	15	0.03	0.82 [0.64;0.99]	0.18	1.05 [0.98;1.12]	0.27	1.04 [0.97;1.10]	0.03	0.83 [0.69;0.98]	0.08	1.06 [0.99;1.13]	0.35	1.05 [0.95;1.15]
	NAT2	33	0.64	1.01 [0.96;1.07]	0.09	1.04 [0.99;1.09]	2.8E-3	0.88 [0.80;0.96]	0.08	0.96 [0.92;1.00]	0.04	0.92 [0.84;1.00]	0.01	1.04 [1.01;1.06]
	SOD2	8	0.42	1.06 [0.91;1.21]	0.21	0.97 [0.92;1.02]	0.05	1.13 [1.00;1.26]	0.05	0.96 [0.92;1.00]	0.33	1.04 [0.96;1.13]	0.28	0.98 [0.93;1.02]
DNA repair	XPC	10	0.06	1.15 [0.99;1.30]	0.37	0.98 [0.92;1.03]	0.08	0.96 [0.92;1.00]	0.02	1.05 [1.01;1.10]	0.07	0.92 [0.82;1.01]	0.05	1.06 [1.00;1.13]
	XRCC3	10	2.3E-3	1.25 [1.09;1.41]	0.14	0.86 [0.67;1.05]	0.24	1.06 [0.96;1.15]	0.22	0.97 [0.93;1.02]	0.29	1.02 [0.98;1.07]	0.04	0.90 [0.81;0.99]

We used the lowest *P* value obtained for SNPs in one gene to show the significance of the association between a candidate gene and skin aging trait. *P* values were adjusted for multiple testing by Bonferroni correction. Here, we corrected for the number of SNPs (N = 509) used in the analysis.
Significant associations with an adjusted *P* value < 0.05 (raw *P* value < 9.8E-05) are printed in bold and underlined.
SNP Number: number of included SNPs per gene.

Acknowledgments

We thank all field workers and study participants.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2016.12.017.

References

[1] G.J. Fisher, S. Kang, J. Varani, Z. Bata-Csorgo, Y. Wan, S. Datta, J.J. Voorhees, Mechanisms of photoaging and chronological skin aging, Arch. Dermatol. 138 (2002).
[2] A. Vierkötter, U. Kramer, D. Sugiri, A. Morita, A. Yamamoto, N. Kaneko, M. Matsui, J. Krutmann, Development of lentigines in German and Japanese women correlates with variants in the SLC45A2 gene, J. Invest. Dermatol. 132 (2012) 733–736.
[3] A.L. Chang, G. Atzmon, A. Bergman, S. Brugmann, S.X. Atwood, H.Y. Chang, N. Barzilai, Identification of genes promoting skin youthfulness by genome-wide association study, J. Invest. Dermatol. 134 (2014) 651–657.
[4] M. Bastiaens, The melanocortin-1-receptor gene is the major freckle gene, Hum. Mol. Genet. 10 (2001) 1701–1708.
[5] A. Elfakir, K. Ezzedine, J. Latreille, L. Ambroisine, R. Jdid, P. Galan, S. Hercberg, F. Gruber, D. Malvy, E. Tschachler, et al., Functional MC1R-gene variants are associated with increased risk for severe photoaging of facial skin, J. Invest. Dermatol. 130 (2010) 1107–1115.
[6] S. Le Clerc, L. Taing, K. Ezzedine, J. Latreille, O. Delaneau, T. Labib, C. Coulonges, A. Bernard, S. Melak, W. Carpentier, et al., A genome-wide association study in Caucasian women points out a putative role of the STXBP5L gene in facial photoaging, J. Invest. Dermatol. 133 (2013) 929–935.
[7] L.C. Jacobs, M.A. Hamer, D.A. Gunn, J. Deelen, J.S. Lall, D. van Heemst, H.W. Uh, A. Hofman, A.G. Uitterlinden, C.E. Griffiths, et al., A genome-wide association study identifies the skin color genes IRF4, MC1R, ASIP, and BNC2 influencing facial pigmented spots, J. Invest. Dermatol. 135 (2015) 1735–1742.
[8] F. Liu, M.A. Hamer, J. Deelen, J.S. Lall, L. Jacobs, D. van Heemst, P.G. Murray, A. Wollstein, A.J. de Craen, H.W. Uh, et al., The MC1R gene and youthful looks, Curr. Biol. 26 (2016) 1213–1220.
[9] X. Wang, M. Lu, J. Qian, Y. Yang, S. Li, D. Lu, S. Yu, W. Meng, W. Ye, L. Jin, Rationales, design and recruitment of the Taizhou Longitudinal study, BMC Public Health 9 (2009) 223.
[10] A. Vierkötter, U. Ranft, U. Krämer, D. Sugiri, V. Reimann, J. Krutmann, The SCINEXA: A novel, validated score to simultaneously assess and differentiate between intrinsic and extrinsic skin ageing, J. Dermatol. Sci. 53 (2009) 207–211.
[11] M.Z. Li, A. Vierkötter, T. Schikowski, A. Huls, A.A. Ding, M.S. Matsui, B.W. Deng, C. Ma, A.G. Ren, J. Zhang, et al., Epidemiological evidence that indoor air pollution from cooking with solid fuels accelerates skin aging in Chinese women, J. Dermatol. Sci. 79 (2015) 148–154.
[12] P.J. Kersey, J.E. Allen, I. Armean, S. Boddu, B.J. Bolt, D. Carvalho-Silva, M. Christensen, P. Davis, L.J. Falin, C. Grabmueller, et al., Ensembl Genomes 2016: more genomes more complexity, Nucleic Acids Res. 44 (2016) D574–580.
[13] P.A. Harper, J.M.Y. Wong, M.S.M. Lam, A.B. Okey, Polymorphisms in the human AH receptor, Chem. Biol. Interact. 141 (2002) 161–187.
[14] Y. Chen, Y. Bai, J. Yuan, W. Chen, J. Sun, H. Wang, H. Liang, L. Guo, X. Yang, H. Tan, et al., Association of polymorphisms in AhR, CYP1A1, GSTM1, and GSTT1 genes with levels of DNA damage in peripheral blood lymphocytes among coke-oven workers, Cancer Epidemiol. Biomarkers Prev. 15 (2006) 1703–1707.
[15] A. Wang, M.J. Shrubsole, J.M. Rice, Q. Cai, M.A. Doll, J. Long, W.E. Smalley, Y. Shyr, R. Sinha, R.M. Ness, et al., Meat intake, heterocyclic amine exposure, and metabolizing enzyme polymorphisms in relation to colorectal polyp risk, Cancer Epidemiol. Biomarkers Prev. 17 (2008) 320–329.
[16] W.T. Hung, G.H. Lambert, P.W. Huang, D.G. Patterson Jr., Y.L. Guo, Genetic susceptibility to dioxin-like chemicals' induction of cytochrome P4501A2 in

the human adult linked to specific AhRR polymorphism, Chemosphere 90 (2013) 2358–2364.
[17] N.L. Nock, D. Tang, A. Rundle, C. Neslund-Dudas, A.T. Savera, C.H. Bock, K.G. Monaghan, A. Koprowski, N. Mitrache, J.J. Yang, et al., Associations between smoking, polymorphisms in polycyclic aromatic hydrocarbon (PAH) metabolism and conjugation genes and PAH-DNA adducts in prostate tumors differ by race, Cancer Epidemiol. Biomarkers Prev. 16 (2007) 1236–1245.
[18] L.C. Jacobs, A. Wollstein, O. Lao, A. Hofman, C.C. Klaver, A.G. Uitterlinden, T. Nijsten, M. Kayser, F. Liu, Comprehensive candidate gene study highlights UGT1A and BNC2 as new genes determining continuous skin color variation in Europeans, Hum. Genet. 132 (2013) 147–158.
[19] N. Eriksson, J.M. Macpherson, J.Y. Tung, L.S. Hon, B. Naughton, S. Saxonov, L. Avey, A. Wojcicki, I. Pe'er, J. Mountain, Web-based, participant-driven studies yield novel genetic associations for common traits, PLoS Genet. 6 (2010) e1000993.
[20] A. Hatamochi, T. Hamada, M. Yoshino, T. Hashimoto, The first Japanese case of the arthrochalasia type of Ehlers-Danlos syndrome with COL1A2 gene mutation, Gene 538 (2014) 199–203.
[21] F. Malfait, S. Symoens, N. Goemans, Y. Gyftodimou, E. Holmberg, V. Lopez-Gonzalez, G. Mortier, S. Nampoothiri, M.B. Petersen, A. De Paepe, Helical mutations in type I collagen that affect the processing of the amino-propeptide result in an Osteogenesis Imperfecta/Ehlers-Danlos Syndrome overlap syndrome, Orphanet. J. Rare Dis. 8 (2013) 78.
[22] U. Schwarze, R. Hata, V.A. McKusick, H. Shinkai, H.E. Hoyme, R.E. Pyeritz, P.H. Byers, Rare autosomal recessive cardiac valvular form of Ehlers-Danlos syndrome results from mutations in the COL1A2 gene that activate the nonsense-mediated RNA decay pathway, Am. J. Hum. Genet. 74 (2004) 917–930.
[23] A. De Paepe, F. Malfait, The Ehlers-Danlos syndrome, a disorder with many faces, Clin. Genet. 82 (2012) 1–11.