Facial wrinkles in Europeans: a genome-wide association study

M.A. Hamer
L.M. Pardo Cortes
L.C. Jacobs
J. Deelen
A.G. Uitterlinden
E. Slagboom
D. van Heemst
H.W. Uh
M. Beekman
M. Kayser
F. Liu
D.A. Gunn
T. Nijsten

J Invest Dermatol. 2018 Aug;138(8):1877-1880

To the Editor,

Wrinkles are among the most notable components of skin aging and are influenced by many different risk factors[1]. Although wrinkle variation has been shown to be a heritable trait, (55%)[2], specific gene variants for wrinkles have not yet been identified. Previous studies have identified the *MC1R* gene as influencing skin photoaging and pigmented spots[3-6], but its role in wrinkling is not clear. In this study, we performed the largest genome-wide association study (GWAS) for global facial wrinkles available to date in 3513 participants from the Rotterdam Study (RS) using a digital wrinkle measure[1] and sought to replicate the most suggestive associations in an independent dataset of 599 participants from the Leiden Longevity Study (LLS).

A detailed description of the methods is presented in the Supplementary Material. The RS is an ongoing Dutch prospective population-based cohort study of 14,926 participants aged 45 years or older[7]. This study includes 3513 northwestern European participants for whom standardized facial photographs and quality-controlled genotype data were available. The RS has been approved by the Medical Ethics Committee of the Erasmus University Medical Center 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. The LLS is a family-based study[8] that includes 599 participants in this study. In the RS, wrinkle area was digitally quantified as wrinkle area percentage of the face using semi-automated image analysis of high-resolution facial photographs. For wrinkle grading in the LLS, a 9-point photonumeric scale was used[2]. The study protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all participants gave written informed consent. In the RS, DNA from whole blood was extracted following standard protocols, and quality controls were applied on markers and individuals[7]. Imputations were performed with 1000 Genomes (GIANT phase I version 3) as the reference panel[9]. In total, 30,072,738 markers were genotyped/imputed. After quality controls, 9,009,554 autosomal single-nucleotide polymorphisms (SNPs) were available. In the LLS, imputation was performed similarly, and association testing was conducted using QT-assoc[10]. The RS served as discovery dataset. We performed linear regression using an additive model (SNP dosage data)[11] adjusting for age, sex, the first four genetic principal components, and two technical variables. These last two variables correct for possible variations in resolution and flash light of the facial photos[1]. 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[4]. We selected all SNPs with P-values $<5\times10^{-6}$ for the replication phase. We also performed a meta-analysis of the RS and LLS together for the top hits, as well as a genome-wide meta-analysis. Several sensitivity analyses (top SNP associations in men and women separately; with different facial wrinkling sites; possible interactions between SNPs and sex, body mass index, and smoking; and a univariate analysis excluding age and sex) and validation of previously published associations between SNPs and skin aging were performed (Supplementary Material).

In the RS, most participants were women (N=2045, 58.2%) and the median age was 66.2 (overall: range 51-98; men: median 66.5, range 51-96; women: median 66.0, range 51-98) years. Men showed a higher average wrinkle area (median facial wrinkle area 4.4%, interquartile range (IQR) 2.9-6.2) than women (3.5%, IQR 2.1-5.5). In the LLS, the mean age was 63.1 years, and 53.8% of participants were women (Supplementary Table S1). The GWAS of global facial wrinkle area in the RS yielded 25 suggestive hits (P-values $<5\times10^{-6}$, Table 1), but none of them were genome-wide significant (Figure 1, Supplementary Figures S1 and S2). The strongest signal was found for an intergenic SNP (rs10476781; P-value 9.5×10^{-8}) on chromosome 5 between the Neuromedin U Receptor 2 (*NMUR2*) and *CTB-1202.1* (long non-coding RNA, *LINC01933*) genes. In the RS, this SNP had a minor allele frequency of 6% and an imputation score of 0.5. The SNP rs10476781 showed moderate linkage disequilibrium (LD, r^2=0.4) with other SNPs on chromosome 5, explaining the moderate imputation score. The effect allele (rs10476781[T], allele frequency 94%) had an effect size of -0.21 (standard error 0.04).

Estimating pairwise LD between all SNPs with suggestive associations (25 SNPs, Table 1) resulted in 11 independent loci (r^2 ≤0.5). There was no LD between rs10476781 and other suggestive SNPs in our dataset (r^2 ≤0.5, Supplementary Table S2 and Supplementary Figure S3). We tested for associations between wrinkles in the LLS replication cohort and the 25 SNPs with suggestive associations. The top SNP, rs10476781, had a nominal P-value of 0.08 in the LLS, and the others could not be replicated (all P-values > 0.2). In a meta-analysis of the two cohorts for the top hits, rs10476781 was genome-wide significant (P-value 2.2×10^{-8}, Table 1). Other suggestive associations (P-values $<=5\times10^{-6}$) from the genome-wide meta-analysis of the two cohorts are presented in Supplementary Table S3 and Supplementary Figure S4. Additional genome-wide meta-analysis of the RS and LLS did not yield any new findings (Supplementary Material, including Supplementary Table S3).

Because of known sex differences in facial wrinkling[1], we also tested for associations between the top SNPs and global wrinkling in a sex-stratified analysis. No genome-wide significant hits or interactions (SNP*sex) were found (Supplementary Table S4).

To our knowledge, this is the largest GWAS of global facial wrinkling conducted thus far, and we found that the rs10476781 SNP was a suggestive hit for global facial wrinkling in the RS (3513 northwestern Europeans) and a significant genome-wide hit in a meta-analysis of the RS and LLS cohorts together (N=4122). However, we cannot exclude that this may be a false positive finding because the imputation score in the RS was moderate, and the SNP has a very low frequency in the general population (minor allele frequency <0.01, and thus it was not included in the latest release of 1000 Genomes). The latter likely explains the moderate imputation quality because rare variants are more difficult to impute. However, it has a higher frequency in Dutch populations (GoNL, a Dutch-specific reference dataset; 2% minor allele frequency, although with low quality[12]), and, among the replicated SNPs in the LLS cohort, this SNP had the lowest P-value. Further confirmation of the association of this SNP with wrinkles is now required.

Table 1. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles in the Rotterdam Study and Leiden Longevity Study and a meta-analysis of these two cohorts[a]

			Discovery cohort: RS (N=3513)						Replication cohort: LLS (N=599)				Meta-analysis: RS & LLS (N=4112)						
SNP	**Chr**	**Position[b]**	**EA**	**OA**	**EAF**	**OAF**	**β (SE)**	**P-value**	**EA**	**EAF**	**β (SE)**	**P-value**	**EA**	**Dir**	**Z[c]**	**P-value**	**I²**	**Cochran's Q**	**Het P-value**
1:3118674:D	1	3118674	D	I	0.12	0.88	0.11 (0.02)	1.8×10^{-6}	I	0.90	0.18 (0.13)	0.18	D	+-	3.90	9.7×10^{-5}	89.40	9.40	0.002
rs11577655	1	3119489	T	C	0.13	0.87	0.11 (0.02)	4.6×10^{-6}	C	0.90	0.17 (0.13)	0.19	T	+-	3.74	1.9×10^{-4}	88.60	8.79	0.003
rs6429657	1	14702354	A	G	0.96	0.04	-0.19 (0.04)	1.6×10^{-6}	G	0.05	0.17 (0.18)	0.35	A	–	-4.79	1.7×10^{-6}	0	0.95	0.33
rs702491	1	54194992	T	C	0.19	0.81	0.09 (0.02)	2.4×10^{-6}	T	0.21	0.09 (0.09)	0.33	T	++	4.74	2.1×10^{-6}	0	0.78	0.38
rs61812508	1	147251772	A	G	0.05	0.95	-0.18 (0.04)	4.3×10^{-6}	G	0.96	0.08 (0.20)	0.69	A	–	-4.40	1.1×10^{-5}	48.20	1.93	0.16
rs11583958	1	147291718	A	T	0.04	0.96	-0.18 (0.04)	3.3×10^{-6}	T	0.96	-0.04 (0.19)	0.84	A	-+	-4.22	2.4×10^{-5}	73.90	3.83	0.05
1:246689691:I	1	246689691	D	I	0.60	0.40	0.07 (0.02)	3.7×10^{-6}	D	0.59	-0.05 (0.07)	0.54	D	+-	4.05	5.2×10^{-5}	81.50	5.42	0.02
rs114667268	2	12433490	T	C	0.01	0.99	-0.49 (0.10)	2.9×10^{-6}	C	0.99	-0.44 (0.65)	0.49	T	-+	-4.07	4.8×10^{-5}	82.90	5.84	0.02
rs7608236	2	180062867	A	G	0.29	0.71	-0.07 (0.02)	4.1×10^{-6}	G	0.72	-0.06 (0.08)	0.43	A	-+	-3.96	7.6×10^{-5}	83.90	6.20	0.01
rs116248825	3	26420135	A	C	0.04	0.96	-0.28 (0.06)	4.1×10^{-6}	C	0.96	0.28 (0.25)	0.27	A	–	-4.68	2.9×10^{-6}	0	0.55	0.46
rs9867656	3	30100084	A	G	0.34	0.66	-0.07 (0.01)	3.7×10^{-6}	A	0.35	-0.06 (0.07)	0.37	A	–	-4.62	3.9×10^{-6}	0	0.89	0.35
rs11711327	3	30101254	A	G	0.66	0.34	0.07 (0.01)	3.1×10^{-6}	G	0.35	-0.06 (0.07)	0.38	A	++	4.65	3.3×10^{-6}	0	0.93	0.34
rs112608607	5	102908739	T	C	0.97	0.03	0.22 (0.05)	3.8×10^{-6}	T	0.97	0.21 (0.22)	0.35	T	++	4.63	3.7×10^{-6}	0	0.83	0.36
rs113322056	5	102913288	A	G	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	A	0.96	0.18 (0.21)	0.41	A	++	4.64	3.4×10^{-6}	3.60	1.04	0.31
rs146551307	5	102915236	T	C	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	T	0.96	0.18 (0.21)	0.42	T	++	4.64	3.5×10^{-6}	3.80	1.04	0.31
5:102915644:D	5	102915644	D	I	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	I	0.96	0.16 (0.21)	0.44	D	–	-4.53	6.0×10^{-6}	6.40	1.07	0.30
rs10476781	**5**	**151763633**	**T**	**C**	**0.94**	**0.06**	**-0.21 (0.04)**	**9.5×10^{-8}**	**T**	**0.94**	**-0.33 (0.19)**	**0.08**	**T**	–	**-5.60**	**2.2×10^{-8}**	**0**	**0.19**	**0.67**
rs72811030	5	179729009	A	G	0.38	0.62	0.07 (0.02)	1.7×10^{-6}	G	0.60	-0.04 (0.08)	0.62	A	++	4.61	4.0×10^{-6}	46.30	1.86	0.17
rs1225927	6	7871037	T	G	0.75	0.25	0.07 (0.02)	3.5×10^{-6}	T	0.75	0.08 (0.08)	0.30	T	++	4.69	2.8×10^{-6}	0	0.67	0.41
9:16847398:D	9	16847398	D	I	0.98	0.02	0.30 (0.07)	4.7×10^{-6}	I	0.02	-0.13 (0.31)	0.68	D	++	4.39	1.1×10^{-5}	46.40	1.86	0.17
rs185291539	10	84338421	A	G	0.98	0.02	0.41 (0.09)	4.8×10^{-6}	A	0.97	0.03 (0.26)	0.90	A	++	4.28	1.9×10^{-5}	62.20	2.64	0.10
rs62047859	16	76826391	A	T	0.03	0.97	0.21 (0.04)	1.0×10^{-6}	T	0.97	-0.26 (0.24)	0.29	A	++	4.92	8.9×10^{-7}	0	0.80	0.37
rs62077967	17	61253263	C	G	0.96	0.04	0.19 (0.04)	4.6×10^{-6}	C	0.96	-0.05 (0.19)	0.81	C	+-	4.15	3.4×10^{-5}	74.10	3.87	0.05
rs72845240	17	61361539	C	G	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	G	0.96	-0.06 (0.19)	0.77	C	-+	-4.12	3.8×10^{-5}	75.50	4.08	0.04
rs189819077	18	34933012	A	G	0.03	0.97	-0.20 (0.04)	1.8×10^{-6}	G	0.97	0.15 (0.23)	0.51	A	–	-4.67	3.0×10^{-6}	32.90	1.49	0.22

Abbreviations: A, adenine; C, cytosine; Chr, chromosome; D, deletion; Dir, direction of the effects; EA, effect allele; EAF, effect allele frequency; G, guanine; GWAS, genome-wide association study; Het P-value, heterogeneity P-value; I, insertion; I^2, heterogeneity I^2; LLS, Leiden Longevity Study; OA, other allele; OAF, other allele frequency; RS, Rotterdam Study; SE, standard error; SNP, single-nucleotide polymorphism; T, thymine. Boldface indicates the top SNP. [a]analyses are adjusted for age, sex, and the first four genetic principal components and in addition, for the RS, for technical variables of the digital measurement; [b]based on GRCh37/hg19; [c]weighted Z-score.

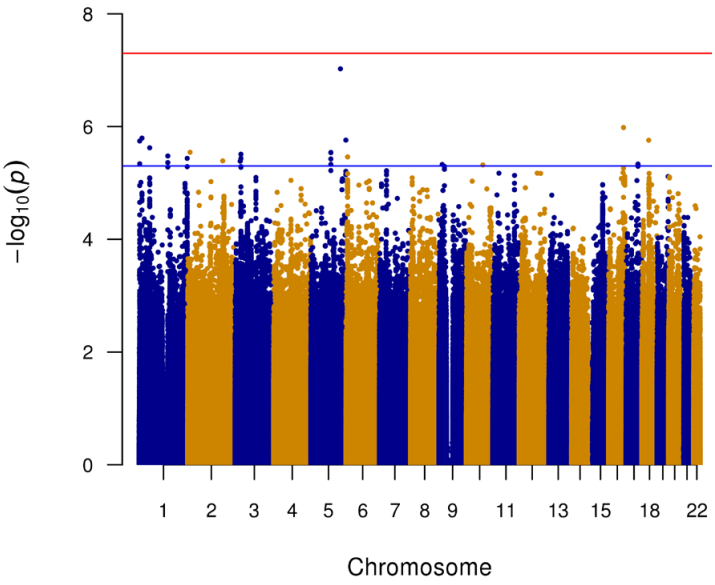


Figure 1. Manhattan plot of the genome-wide associations for wrinkle area in the discovery cohort (Rotterdam Study, N=3513). All SNPs are represented by dots and displayed per chromosome (x-axis); y-axis shows negative $\log_{10}$-transformed P-values.

The *MC1R* gene influences skin aging[3,5,6,13]. However, we did not find any significant association between *MC1R* variants and wrinkles, which suggests that these variants are not influencing facial wrinkle variation as measured in the RS cohort but instead other skin aging phenotypes, such as pigmented age spots[4]. Furthermore, we did not replicate SNPs previously reported as associated with skin aging, except for a nominally significant association between rs12203592 and wrinkles in the LLS. Reasons for the lack of association could be that these SNPs are false positives because of the small sample sizes[14] or because of phenotypic heterogeneity in photoaging versus wrinkling in our study. In addition, genetic heterogeneity could play a role.

We cannot exclude that other SNPs may be associated with wrinkling, because the heritability was 42% in the RS (P-value 4.4×10^{-8}, 95%CI 28% to 61%)[15]. Most likely, the effects of each influencing SNP are too small to be detected with a sample size as used in this study, because we had a 77% power to detect SNPs with moderate effects (Supplementary Material including Supplementary Tables S5-S8). This highlights the importance of increasing sample sizes for future GWASs. Another limitation is that in the replication cohort, only photonumeric grading was available, although there is a high correlation between digital and photonumeric grading (Spearman's rho 0.8-0.9)[16]; hence, we believe that our replication is valid.

In conclusion, we found a genome-wide statistically significant association between the SNP rs10476781 (P-value 2.2×10^{-8}) and global facial wrinkling in a meta-analysis of two independent northwestern European cohorts. This intergenic SNP (628 kilo base pairs downstream of the Neuromedin U Receptor gene) is an interesting candidate but needs further validation.

ACKNOWLEDGMENTS

The authors are grateful to the study participants, the staff from the Rotterdam Study and the Leiden Longevity Study, and the participating general practitioners and pharmacists. Regarding the Rotterdam Study, we thank Sophie Flohil, Emmilia Dowlatshahi, Robert van der Leest, Joris Verkouteren and Ella van der Voort for collecting the phenotypes. Additionally we thank Sophie van den Berg for masking and reviewing all the photographs. We acknowledge Jaspal Lall for masking the photographs and creating the digital wrinkle measurements.

REFERENCES

1. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M, et al. Lifestyle and Physiological Factors Associated with Facial Wrinkling in Men and Women. *J Invest Dermatol*. 2017;137(8):1692-9.
2. Gunn DA, Rexbye H, Griffiths CE, Murray PG, Fereday A, Catt SD, et al. Why some women look young for their age. *PLoS One*. 2009;4(12):e8021.
3. Elfakir A, Ezzedine K, Latreille J, Ambroisine L, Jdid R, Galan P, et al. Functional MC1R-gene variants are associated with increased risk for severe photoaging of facial skin. *J Invest Dermatol*. 2010;130(4):1107-15.
4. Jacobs LC, Hamer MA, Gunn DA, Deelen J, Lall JS, van Heemst D, et al. A Genome-Wide Association Study Identifies the Skin Color Genes IRF4, MC1R, ASIP, and BNC2 Influencing Facial Pigmented Spots. *J Invest Dermatol*. 2015;135(7):1735-42.
5. Liu F, Hamer MA, Deelen J, Lall JS, Jacobs L, van Heemst D, et al. The MC1R Gene and Youthful Looks. *Curr Biol*. 2016;26(9):1213-20.
6. Suppa M, Elliott F, Mikeljevic JS, Mukasa Y, Chan M, Leake S, et al. The determinants of periorbital skin ageing in participants of a melanoma case-control study in the U.K. *Br J Dermatol*. 2011;165(5):1011-21.
7. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol*. 2015;30(8):661-708.
8. Westendorp RG, van Heemst D, Rozing MP, Frolich M, Mooijaart SP, Blauw GJ, et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. *J Am Geriatr Soc*. 2009;57(9):1634-7.
9. Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491(7422):56-65.
10. Uh HW, Beekman M, Meulenbelt I, Houwing-Duistermaat JJ. Genotype-Based Score Test for Association Testing in Families. *Stat Biosci*. 2015;7(2):394-416.
11. Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. 2010;11:134.
12. Boomsma DI, Wijmenga C, Slagboom EP, Swertz MA, Karssen LC, Abdellaoui A, et al. The Genome of the Netherlands: design, and project goals. *Eur J Hum Genet*. 2014;22(2):221-7.
13. Law MH, Medland SE, Zhu G, Yazar S, Vinuela A, Wallace L, et al. Genome-Wide Association Shows that Pigmentation Genes Play a Role in Skin Aging. *J Invest Dermatol*. 2017;137(9):1887-94.
14. Ioannidis JP. Genetic associations: false or true? *Trends Mol Med*. 2003;9(4):135-8.
15. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42(7):565-9.
16. Hamer MA, Jacobs LC, Lall JS, Wollstein A, Hollestein LM, Rae AR, et al. Validation of image analysis techniques to measure skin aging features from facial photographs. *Skin Res Technol*. 2015;21(4):392-402.

SUPPLEMENTARY MATERIAL

MATERIAL AND METHODS

Study population

Rotterdam Study (RS)

The RS is an ongoing prospective population-based cohort study of 14,926 participants aged 45 years or older, living in Ommoord, a suburb of Rotterdam, the Netherlands. The RS consists of a major cohort (RS-I, N=7983) and two extensions (RS-II, N=3011 and RS-III, N=3932). Details of the study design have been described elsewhere[1]. The current study includes 3513 participants of northwestern European ancestry (who visited the research center between September 2010 and June 2014), for whom facial photographs and genotype data were available after quality controls. During routine visits at the research center, a full body skin examination was performed by trained physicians, and high-resolution standardized full-face photographs were obtained of participants not wearing make-up, cream, or jewelry, using a Premier 3dMD face3-plus UHD camera (3dMD, Atlanta, GA, USA) in a room without daylight.

Leiden Longevity Study

The LLS has been described in detail previously[2]. This family-based study consists of 1671 offspring of 421 nonagenarian sibling pairs of Dutch descent and their 744 partners[3]. For the current study, there were 599 participants with valid genotype data after quality control and a suitable facial photograph (exclusions were for example due to the presence of beards or poor photo quality). During visits at the Leiden University Medical Center, high-resolution standardized full-face photographs were obtained of participants not wearing make-up, cream, or jewelry, using a Fuji S2 (Tokyo, Japan) camera system. The photos used in this study were collected from November 2006 to April 2008.

Phenotyping

In the RS, wrinkle area was digitally quantified as wrinkle area percentage of the facial skin using semi-automated image analysis of the high-resolution facial photographs. The algorithms, digital rendering, measurement and validation of the outcome measure have been described in detail previously, using a randomly selected subset of photographs of 100 participants[4]. To correct for technical variation, we included two variables in the analyses: the first accounted for differences in resolution between two sets of the images and the second accounted for variations in flash light[5].

For wrinkle grading in the LLS, individuals were graded by two skin aging experts. Skin wrinkling was graded on a 9-point scale by a visual assessment of the number and depth of fine and course wrinkles (i.e. shallow indentations or lines, deep lines, furrows or creases). Fine wrinkles tend to

appear in periorbital and perioral regions and had a small weighting in the 9-point photographic scale; coarse wrinkles had a strong weighting in the scale and tend to appear on the forehead, glabella, chin, nasolabial and periorbital areas and are larger and closer to the eyes and mouth than are fine wrinkles. The mean value from the two graders was used for further analyses[6].

Genotyping and Imputation

In the RS, DNA from whole blood was extracted following standard protocols with details presented elsewhere[1]. In brief, genotyping was carried out using the Infinium II HumanHap 550K Genotyping BeadChip version 3 (Illumina, San Diego, CA, USA) for RS-I (N=6291) and RS-II (N=2157) cohorts while Illumina Human 610 Quad Arrays were used to genotype the RS-III cohort (N=3048). A quality control on markers and individuals was applied to the genotyping[1]. We imputed the RS-I, RS-II and RS-III cohorts separately, with 1000Genomes (GIANT Phase I version 3) as the reference panel[7]. In total, 30,072,738 markers were genotyped and/or imputed. After all quality controls, a total of 9,009,554 autosomal SNPs (minor allele frequency >1%; imputation quality score (r^2_pihat) > 0.3) was available.

In the LLS, genotyping was performed using Illumina Human660W-Quad and OmniExpress BeadChips as described elsewhere[8]. Imputation was performed using IMPUTE with the 1000 Genomes (Phase I version 3) reference panel[7]. Association testing was conducted using QT-assoc[9] to take into account family relations and imputation uncertainty.

Statistical analysis

Stage 1: Discovery phase

In the RS, the wrinkle area distribution was highly right-skewed. Therefore, wrinkle area was transformed using the natural logarithm (ln). We used the three cohorts from the RS as the discovery dataset. The associations between SNPs and (ln-transformed) wrinkles were tested per cohort. We performed linear regression using an additive model using SNP dosage data[10] and adjusting for age, sex, the first four genetic principal components, resolution variability and flash light variability[5]. The significance of the association was tested using the likelihood ratio test with one degree of freedom. The GWAS analyses were implemented in the ProbABEL package[10]. The quality control of the GWAS summary statistics per cohort was performed using the EasyQC software with parameter defaults[11]. Next, a meta-analysis of the three RS cohorts was carried out using the METAL software using an inverse variance approach, allowing for genomic control correction (genomic inflation factors were <1.03, therefore no adjustments were made) and heterogeneity checks[12]. P-values < 5×10^{-8} were considered genome-wide significant.

Stage 2: Replication and joint meta-analysis

We selected all SNPs with P-values <5×10^{-6} from the discovery phase to test for associations between these SNPs and wrinkles in the LLS cohort. The SNPs selected for replication were analyzed in the LLS cohort using linear regression adjusted for age, sex, and familial relations using the

software package QT-assoc[9], which is based on a modified version of the score test. To correct for multiple testing, we calculated the pair-wise linkage disequilibrium (LD; r^2) between the SNPs with suggestive association in plink using genotypes from BestGuess data[13]. The P-values were adjusted by dividing the nominal P-value by the number of independent tests. We identified 11 independent loci ($r^2 \leq 0.5$), leading to a significance threshold of 4.5×10^{-3}. We also performed a meta-analysis of the RS and LLS together for the top hits, using the METAL software mentioned above. P-values < 5×10^{-8} were considered as genome-wide significant using the weighted Z-score method, implemented in METAL[12]. SNP heterogeneity was tested using I^2 and Cochran's Q methods. The top SNPs were annotated to genes using Ensembl (http://browser.1000genomes.org/index.html).

Sensitivity analyses in the RS

Because the top SNP we found in our GWAS has been associated with body weight in other studies[14,15], and given the known associations between BMI and wrinkles[16], we also tested associations between the top SNPs identified in the RS and global wrinkles by additionally adjusting for BMI to determine whether the effects were independent of BMI. We have also added analyses with BMI*SNP as an interaction term for the top SNPs, as well as analyses with smoking*SNP. In addition, we tested for interactions between the suggestive SNPs and sex (by adding a sex*SNP covariate) and performed a sex-stratified analysis to analyze the effects of the suggestive SNPs (P-values $<5 \times 10^{-6}$) separately in men and women, because it is known that wrinkle prevalence is different between sexes[5]. Furthermore, we have also performed a univariate analysis (without age and sex as covariates, but including the two technical variables and the first four genetic principal components). Data on site-specific wrinkling, namely crow's feet, forehead wrinkles and upper lip wrinkles (in women only) was also available[5]. The technical wrinkle measurements were the same as for the global facial wrinkles and area of wrinkles was measured. We investigated whether the suggestive SNPs that were associated with global wrinkling also associate with site-specific wrinkling. Finally, we have also performed a genome-wide meta-analysis, including 4112 subjects from the RS and the LLS.

Validation of previously published associations between SNPs and skin aging

To check the associations of recently found candidate SNPs for skin aging we examined these SNPs in our dataset. The following SNPs, which showed a genome-wide significant association in previous studies, were investigated: rs6975107, rs11863929, rs7616661[17], rs322458[18], rs12203592, rs62543565, rs35063026, rs6059655[19], rs11876749[20], rs185146, rs12203592, rs4268748, rs1805007 and rs1805008[21]. We were unable to determine the effect of rs318125, rs5916727 and rs1578826[17], because we only analyzed autosomal SNPs.

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[5]). 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-hand side of the face[4].

Statistical analysis

The site-specific facial wrinkles followed a right-skewed distribution, but also included a considerable proportion of zeros as value for some photos, indicating there were no wrinkles at the 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[22]. We tested for associations between SNPs and site-specific wrinkles using the same methods as for global wrinkles (main manuscript).

RESULTS

Study populations

Rotterdam Study

Between September 2010 and June 2014, a total of 4649 participants visited the in-person examination of the RS, which included extensive dermatological assessments and capturing a facial 3D photo. Of these, 818 were excluded due to poor image quality of the 3D photo, non-northwestern European origin, make-up and/or presence of facial hair (e.g. beards). Another 318 participants were excluded because of missing genetic information. Thus, there were 3513 participants eligible for this study. The majority were women (N=2045, 58.2%) and the median age was 66.2 (range 51-98) years old (Supplementary Table S1). Men showed a significant higher average wrinkle area (median facial wrinkle area 4.4%, IQR 2.9 to 6.2) than women (median facial wrinkle area 3.5%, IQR 2.1 to 5.5).

Leiden Longevity Study

The facial grading in the LLS was performed in 660 participants by visual assessment. Of these, 61 were excluded due to missing genotype data after quality control (N=3) or unsuitable photographs (e.g. presence of beards or poor photo quality), leaving 599 individuals for analysis. The mean age of these individuals was 63.1 years and 53.8% were women (Supplementary Table S1).

Stage 1: Discovery phase

The GWAS of global facial wrinkle area in the RS yielded several suggestive hits (P-values $<5\times10^{-6}$), but none of them were genome-wide significant (Figure 1, Supplementary Figures S1 and S2). In total, 25 SNPs with suggestive associations were identified (Table 1). The strongest signal was

found for the SNP rs10476781 (P-value 9.5×10^{-8}) that maps to an intergenic region on chromosome 5 between the gene Neuromedin U Receptor 2 (*NMUR2*) and *CTB-1202.1*, a gene encoding a long non-coding RNA (*LINC01933*). In the RS, this SNP has a minor allele frequency of 6% and an imputation score of 0.5 (the SNP rs10476781 showed moderate LD – r^2 0.4 – with other SNPs on chromosome 5, explaining the moderate imputation score). The effect allele (rs10476781[T], allele frequency 94%) has a β of -0.21 (SE 0.04). We estimated pairwise LD between the SNPs with suggestive associations and considered 11 SNPs as independent loci ($r^2 \leqq 0.5$). There was no LD between rs10476781 and other suggestive SNPs in our dataset ($r^2 \leqq 0.5$, Supplementary Table S2, Supplementary Figure S3).

Stage 2: Replication and joint meta-analysis

We tested for associations between wrinkles in the LLS replication cohort and the 25 SNPs with suggestive associations. One SNP, rs10476781, had a nominal P-value of 0.08 in the LLS, but the others could not be replicated (all P-values >0.2). In the meta-analysis of the two cohorts for the top hits, we found that rs10476781 showed a genome-wide significant P-value of 2.2×10^{-8} (Table 1).

Power calculation

Using the GWAS/QT Power Detection program[23], we found that with the current sample size of N=3513 and the following parameters: a heritability (effect size) of 0.0084 (based on the R^2 of the most significantly associated SNP); a total of 7,000,000 analyzed SNPs; an LD between a SNP and causative genetic variant of 1; 8 covariates and an explained variance of these covariates of 0.27, the power of our study was 0.77.

Sensitivity analyses in the RS

For the most significant SNPs associated with global wrinkles we performed sensitivity analyses by adding BMI as an additional covariate to the model. This analysis showed similar results as our main analysis (top SNP rs10476781: without BMI, Beta -0.21, P-value 9.5×10^{-8}; with BMI, Beta -0.21, P-value 1.37×10^{-7}), indicating that our findings were independent of BMI. Furthermore, we added BMI*SNP in the analyses for the top SNPs (Supplementary Table S5). For the top SNP rs10476781, the interaction term was not significant. For the 25 top SNPs, the interaction term BMI*SNP was significant for only one SNP (BMI*SNP1:246689691:I, P-value=0.01), however not after Bonferroni correction. Thus, we confirm that the effects of the identified SNPs are not mediated through BMI.

For the top SNP rs10476781, the interaction term smoking*BMI was also not significant. For the top 25 SNPs, the interaction term smoking*SNP was significant for two SNPs and former smoking (former smoking*SNP1:3118674.D, P-value=0.02; former smoking*SNPrs11577655, P-value=0.03, Supplementary Table S5), however not after Bonferroni correction.

Because of differences in facial wrinkling between sexes[5], we also tested for associations between the top SNPs and global wrinkling separately for men and women (i.e. sex-stratified analysis). We found that rs10476781 has a considerably stronger effect in women (Beta -0.27, P-value 1.2×10^{-6}) as compared to men (Beta -0.14, P-value 1.5×10^{-2}). However, the interaction term (sex*SNP) was not significant for any of the suggestive SNPs. For the SNPs with suggestive associations besides the top SNP, the effect sizes were more similar for both sexes and in the same direction (Supplementary Table S4). The univariate analysis – excluding age and sex – (Supplementary Table S8) showed similar results, with a P-value for rs10476781 of 1.3×10^{-7}.

Because global wrinkling only has partial correlation with the site-specific wrinkling (correlations between crow's feet, forehead wrinkles and upper lip wrinkles vs. global wrinkles were 0.48, 0.58 and 0.52, respectively[5]), we also tested for associations between the top SNPs associated with global wrinkling and wrinkling at different facial sites: crow's feet, forehead wrinkles and upper lip wrinkles (women only). Of the 25 tested top SNPs for global facial wrinkling, 22 also associated with the various facial wrinkle sites (Supplementary Table S6).

Results of the genome-wide meta-analysis are shown in Supplementary Table S3 and Supplementary Figure S4. Of note, there are multiple significant associations of SNPs that were only present in the LLS. Because of the small sample size in the LLS, these are likely to be false positives.

Validation of previously published associations between SNPs and skin aging

Finally, we performed a look-up of SNPs that have been previously reported to demonstrate associations with facial aging. The SNPs significantly associated with facial aging in the GWAS of 428 Ashkenazi Jews[17] were not available in the RS cohort, except for rs7616661, which showed a non-significant P-value of 0.19 in our meta-analysis (Supplementary Table S7). The SNP rs322458 significantly associated in the GWAS of 502 French middle-aged women[18] with global skin photoaging (rated using a six-grade ordinal scale) as phenotype[24] had a non-significant P-value of 0.38 in our meta-analysis. The skin color SNPs significantly associated with age pigmented spots in a recent GWAS[19] were all non-significantly associated with wrinkling, as was the case for the SNP associated with sagging eyelids[20] (Supplementary Table S7). One SNP (rs12203592) from the meta-analysis of GWAS of the skin aging phenotype micro-topography score of the back of the hand was significant in the LLS (P-value 0.03), however not in the RS nor in the genome-wide meta-analysis of the two cohorts.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table S1. Characteristics of participants of the Rotterdam Study (N=3513) used here for discovery and of the Leiden Longevity Study (N=599), used for replication

Characteristic	RS (N=3513)	LLS (N=599)
Wrinkle area % - median [IQR] in RS; mean (SD) in LLS[a]	3.9 [2.4 – 5.9]	4.6 (1.3)
Sex – number of females (%)	2045 (58.2)	322 (53.8)
Age at photo in years - median [IQR] in RS; mean (SD) in LLS[b]	66.2 [60.6 – 71.0]	63.1 (6.7)
BMI in kg/m² - mean (SD)	27.6 (4.4)	25.5 (3.6)[c]

Abbreviations: IQR, interquartile range; LLS, Leiden Longevity Study; RS, Rotterdam Study; SD, standard deviation. [a]in the RS a digital measure was used, which was non-normally distributed; in the LLS a photonumeric scale (0-8) was used, which was normally distributed; [b]in the RS age was non-normally distributed; in the LLS age was normally distributed; [c]based on 594 individuals.

Supplementary Table S2. Pairwise linkage disequilibrium with $r^2 \leq 0.5$ between the top SNPs (P-values $<5\times10^{-6}$) associated with global facial wrinkles in the Rotterdam Study

SNP	Chr	Position[a]	Number of SNPs in LD	Left border position	Right border position	KB span	SNPs in LD
1:3118674:D	1	3118674	1	3118674	3119489	0.816	rs11577655
rs11577655	1	3119489	1	3118674	3119489	0.816	1:3118674:D
rs6429657	1	14702354	0	14702354	14702354	0.001	none
rs702491	1	54194992	0	54194992	54194992	0.001	none
rs61812508	1	147251772	1	147251772	147291718	39.947	rs11583958
rs11583958	1	147291718	1	147251772	147291718	39.947	rs61812508
1:246689691:I	1	246689691	0	246689691	246689691	0.001	none
rs116248825	3	26420135	0	26420135	26420135	0.001	none
rs9867656	3	30100084	1	30100084	30101254	1.171	rs11711327
rs11711327	3	30101254	1	30100084	30101254	1.171	rs9867656
rs112608607	5	102908739	3	102908739	102915644	6.906	rs113322056|rs146551307|5:102915644:D
rs113322056	5	102913288	3	102908739	102915644	6.906	rs112608607|rs146551307|5:102915644:D
rs146551307	5	102915236	3	102908739	102915644	6.906	rs112608607|rs113322056|5:102915644:D
5:102915644:D	5	102915644	3	102908739	102915644	6.906	rs112608607|rs113322056|rs146551307
rs10476781	**5**	**151763633**	**0**	**151763633**	**151763633**	**0.001**	**none**
rs72811030	5	179729009	0	179729009	179729009	0.001	none
rs62077967	17	61253263	1	61253263	61361539	108.277	rs72845240
rs72845240	17	61361539	1	61253263	61361539	108.277	rs62077967

Abbreviations: Chr, chromosome; KB span, kilo base pair span; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism. Boldface indicates the top SNP.
[a]based on GRCh37/hg19.

Supplementary Table S3. Top SNPs (P-values <5×10^{-6}) of the genome-wide meta-analysis[a] for global facial wrinkles in the Rotterdam Study and Leiden Longevity Study

			Meta-analysis (RS & LLS, N=4112)									
SNP	**Chr**	**Position[b]**	**EA**	**OA**	**EAF**	**OAF**	**Dir**	**Z[c]**	**P-value**	**I^2**	**Cochran's Q**	**Het P-value**
rs10476781	**5**	**151763633**	**T**	**C**	**0.94**	**0.06**	**--**	**-5.6**	**2.2×10^{-8}**	**0**	**0.19**	**0.67**
rs11213999	11	111634592	A	C	0.08	0.92	--	-5.04	4.7×10^{-7}	0	0.21	0.65
rs62047859	16	76826391	A	T	0.03	0.97	++	4.92	8.9×10^{-7}	0	0.8	0.37
rs147672305	8	105071112	A	T	0.02	0.98	++	4.91	9.1×10^{-7}	76.9	4.32	0.04
rs78569750	18	34858053	T	G	0.06	0.94	--	-4.88	1.0×10^{-6}	0	0.08	0.78
rs62528382	8	105067863	A	G	0.03	0.97	++	4.83	1.4×10^{-6}	40.8	1.69	0.19
rs6429657	1	14702354	A	G	0.96	0.04	--	-4.79	1.7×10^{-6}	0	0.95	0.33
rs1283106	3	106961498	A	C	0.37	0.63	--	-4.77	1.9×10^{-6}	0	0.01	0.93
rs702491	1	54194992	T	C	0.2	0.8	++	4.74	2.1×10^{-6}	0	0.78	0.38
rs1225927	6	7871037	T	G	0.75	0.25	++	4.69	2.8×10^{-6}	0	0.67	0.41
rs116248825	3	26420135	A	C	0.04	0.96	--	-4.68	2.9×10^{-6}	0	0.55	0.46
rs1299331	3	106965492	A	G	0.62	0.38	++	4.68	2.9×10^{-6}	0	0.13	0.71
rs189819077	18	34933012	A	G	0.03	0.97	--	-4.67	3.0×10^{-6}	32.9	1.49	0.22
rs11711327	3	30101254	A	G	0.65	0.35	++	4.65	3.3×10^{-6}	0	0.93	0.34
rs113322056	5	102913288	A	G	0.96	0.04	++	4.64	3.4×10^{-6}	3.6	1.04	0.31
8:105017098:D	8	105017098	D	I	0.03	0.97	++	4.64	3.4×10^{-6}	22.9	1.3	0.25
rs116873518	9	21005828	C	G	0.02	0.98	--	-4.64	3.4×10^{-6}	0	0.44	0.51
rs1283105	3	106962305	C	G	0.62	0.38	++	4.64	3.5×10^{-6}	0	0.02	0.9
rs146551307	5	102915236	T	C	0.96	0.04	++	4.64	3.5×10^{-6}	3.8	1.04	0.31
rs1150997	12	32070095	A	T	0.72	0.28	--	-4.64	3.5×10^{-6}	0	0.05	0.82
rs184605088	20	24536142	A	C	0.02	0.98	--	-4.64	3.5×10^{-6}	0	0.47	0.49
rs112608607	5	102908739	T	C	0.97	0.03	++	4.63	3.7×10^{-6}	0	0.83	0.36
rs9867656	3	30100084	A	G	0.34	0.66	--	-4.62	3.8×10^{-6}	0	0.89	0.35
10:25869856:D	10	25869856	D	I	0.36	0.64	--	-4.62	3.8×10^{-6}	25.5	1.34	0.25
rs1283108	3	106961285	T	C	0.38	0.62	--	-4.62	3.9×10^{-6}	0	0.01	0.9
rs184880542	8	86293264	T	G	0.02	0.98	++	4.62	3.9×10^{-6}	0	0.2	0.65
rs1283103	3	106962755	A	G	0.62	0.38	++	4.61	4.0×10^{-6}	0	0.04	0.84
rs72811030	5	179729009	A	G	0.38	0.62	++	4.61	4.0×10^{-6}	46.3	1.86	0.17
rs117828793	9	28211487	T	C	0.03	0.97	--	-4.61	4.0×10^{-6}	0	0.12	0.73
rs1283102	3	106962929	A	G	0.38	0.62	--	-4.6	4.3×10^{-6}	0	0.06	0.81
rs382029	6	7870856	A	T	0.27	0.73	--	-4.59	4.3×10^{-6}	0	0.44	0.51
rs1283104	3	106962521	C	G	0.62	0.38	++	4.58	4.7×10^{-6}	0	0.02	0.88

Abbreviations: A, adenine; C, cytosine; Chr, chromosome; D, deletion; Dir, direction of the effects; EA, effect allele; EAF, effect allele frequency; G, guanine; Het P-value, heterogeneity P-value; I^2, heterogeneity I^2; LLS, Leiden Longevity Study; OA, other allele; OAF, other allele frequency; RS, Rotterdam Study; SNP, single-nucleotide polymorphism; T, thymine. Boldface indicates the top SNP.

[a]analyses are adjusted for age, sex, and the first four genetic principal components and in addition, for the RS, for technical variables of the digital measurement. Only SNPs present in both RS and LLS cohorts are displayed; [b]based on GRCh37/hg19; [c]weighted Z-score.

Supplementary Table S4. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles[a] in both sexes in the Rotterdam Study and their effects (β) and P-values tested in men and women separately

			Both sexes (N=3513)							Men (N=1468)		Women (N=2045)	
SNP	Chr	Position[b]	EA	OA	EAF	OAF	β (SE)	P-value	P-value interaction SNP*sex[c]	β (SE)	P-value	β (SE)	P-value
rs10476781	**5**	**151763633**	**T**	**C**	**0.94**	**0.06**	**-0.21 (0.04)**	**9.5×10^{-8}**	**0.12**	**-0.14 (0.06)**	**1.5×10^{-2}**	**-0.27 (0.06)**	**1.2×10^{-6}**
rs185291539	10	84338421	A	G	0.98	0.02	0.41 (0.09)	4.8×10^{-6}	0.48	0.32 (0.13)	1.8×10^{-2}	0.46 (0.12)	1.2×10^{-4}
5:102915644:D	5	102915644	D	I	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	0.92	-0.20 (0.06)	1.2×10^{-3}	-0.18 (0.06)	1.9×10^{-3}
9:16847398:D	9	16847398	D	I	0.98	0.02	0.30 (0.07)	4.7×10^{-6}	0.13	0.16 (0.10)	0.11	0.36 (0.08)	1.5×10^{-5}
rs72845240	17	61361539	C	G	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	6.30×10^{-2}	-0.28 (0.06)	1.1×10^{-6}	-0.14 (0.06)	1.6×10^{-2}
rs11577655	1	3119489	T	C	0.13	0.87	0.11 (0.02)	4.6×10^{-6}	0.69	0.12 (0.04)	5.9×10^{-4}	0.11 (0.03)	6.9×10^{-4}
rs62077967	17	61253263	C	G	0.96	0.04	0.19 (0.04)	4.6×10^{-6}	6.30×10^{-2}	0.28 (0.06)	1.1×10^{-6}	0.14 (0.06)	1.6×10^{-2}
rs61812508	1	147251772	A	G	0.05	0.95	-0.18 (0.04)	4.3×10^{-6}	0.49	-0.17 (0.06)	4.0×10^{-3}	-0.19 (0.05)	2.2×10^{-4}
rs7608236	2	180062867	A	G	0.29	0.71	-0.07 (0.02)	4.1×10^{-6}	0.52	-0.08 (0.02)	2.6×10^{-4}	-0.06 (0.02)	3.6×10^{-3}
rs116248825	3	26420135	A	C	0.04	0.96	-0.28 (0.06)	4.1×10^{-6}	0.42	-0.18 (0.09)	3.2×10^{-2}	-0.34 (0.08)	5.1×10^{-5}
rs112608607	5	102908739	T	C	0.97	0.03	0.22 (0.05)	3.8×10^{-6}	0.98	0.21 (0.07)	1.7×10^{-3}	0.21 (0.07)	1.3×10^{-3}
1:246689691:I	1	246689691	D	I	0.6	0.4	0.07 (0.02)	3.7×10^{-6}	0.6	0.06 (0.02)	9.9×10^{-3}	0.08 (0.02)	9.5×10^{-5}
rs9867656	3	30100084	A	G	0.34	0.66	-0.07 (0.01)	3.7×10^{-6}	0.57	-0.08 (0.02)	2.6×10^{-4}	-0.06 (0.02)	3.6×10^{-3}
rs1225927	6	7871037	T	G	0.75	0.25	0.07 (0.02)	3.5×10^{-6}	0.55	0.07 (0.02)	1.4×10^{-3}	0.07 (0.02)	1.1×10^{-3}
rs11583958	1	147291718	A	T	0.04	0.96	-0.18 (0.04)	3.3×10^{-6}	0.35	-0.16 (0.06)	6.6×10^{-3}	-0.20 (0.05)	9.9×10^{-5}
rs11711327	3	30101254	A	G	0.66	0.34	0.07 (0.01)	3.1×10^{-6}	0.59	0.07 (0.02)	3.1×10^{-4}	0.06 (0.02)	2.8×10^{-3}
rs114667268	2	12433490	T	C	0.01	0.99	-0.49 (0.10)	2.9×10^{-6}	0.26	-0.31 (0.16)	4.9×10^{-2}	-0.58 (0.14)	2.5×10^{-5}
rs113322056	5	102913288	A	G	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	0.96	0.20 (0.06)	1.3×10^{-3}	0.19 (0.06)	1.3×10^{-3}
rs146551307	5	102915236	T	C	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	0.95	0.20 (0.06)	1.3×10^{-3}	0.19 (0.06)	1.4×10^{-3}
rs702491	1	54194992	T	C	0.19	0.81	0.09 (0.02)	2.4×10^{-6}	0.96	0.09 (0.03)	4.6×10^{-4}	0.09 (0.03)	6.3×10^{-4}
1:3118674:D	1	3118674	D	I	0.12	0.88	0.11 (0.02)	1.8×10^{-6}	0.95	0.13 (0.03)	1.4×10^{-4}	0.11 (0.03)	8.4×10^{-4}
rs189819077	18	34933012	A	G	0.03	0.97	-0.20 (0.04)	1.8×10^{-6}	0.76	-0.23 (0.06)	1.1×10^{-4}	-0.19 (0.06)	1.9×10^{-3}

Supplementary Table S4. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles[a] in both sexes in the Rotterdam Study and their effects (β) and P-values tested in men and women separately (continued)

			Both sexes (N=3513)							Men (N=1468)		Women (N=2045)	
SNP	Chr	Position[b]	EA	OA	EAF	OAF	β (SE)	P-value	P-value interaction SNP*sex[c]	β (SE)	P-value	β (SE)	P-value
rs72811030	5	179729009	A	G	0.38	0.62	0.07 (0.02)	1.7×10^{-6}	0.15	0.05 (0.02)	2.7×10^{-2}	0.09 (0.02)	2.3×10^{-5}
rs6429657	1	14702354	A	G	0.96	0.04	-0.19 (0.04)	1.6×10^{-6}	0.88	-0.17 (0.06)	3.4×10^{-3}	-0.23 (0.06)	4.8×10^{-5}
rs62047859	16	76826391	A	T	0.03	0.97	0.21 (0.04)	1.0×10^{-6}	0.93	0.22 (0.06)	3.1×10^{-4}	0.21 (0.06)	4.3×10^{-4}

Abbreviations: A, adenine; C, cytosine; Chr, chromosome; D, deletion; EA, effect allele; EAF, effect allele frequency; G, guanine; GWAS, genome-wide association study; OA, other allele; OAF, other allele frequency; SE, standard error; SNP, single-nucleotide polymorphism; T, thymine. Boldface indicates the top SNP.

[a]analyses are adjusted for age, sex, the first four genetic principal components, and technical variables of the digital measurement; [b]based on GRCh37/hg19; [c]P-value of the interaction term SNP*sex, added as covariate in the linear regression.

Supplementary Table S5. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles[a] in the Rotterdam Study and the P-values of the interaction terms SNP*BMI and SNP*smoking, tested separately in the analyses

			RS (N=3513)								
										P-value interaction SNP*smoking[d]	
SNP	Chr	Position[b]	EA	OA	EAF	OAF	β (SE)	P-value	P-value interaction SNP*BMI[c]	SNP*former smoking	SNP*current smoking
rs10476781	**5**	**151763633**	**T**	**C**	**0.94**	**0.06**	**-0.21 (0.04)**	**9.5×10^{-8}**	**0.25**	**0.64**	**0.50**
rs185291539	10	84338421	A	G	0.98	0.02	0.41 (0.09)	4.8×10^{-6}	0.64	0.76	0.72
5:102915644:D	5	102915644	D	I	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	0.42	0.73	0.80
9:16847398:D	9	16847398	D	I	0.98	0.02	0.30 (0.07)	4.7×10^{-6}	0.65	0.21	0.53
rs72845240	17	61361539	C	G	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	0.54	0.87	0.70
rs11577655	1	3119489	T	C	0.13	0.87	0.11 (0.02)	4.6×10^{-6}	0.76	0.03	0.11
rs62077967	17	61253263	C	G	0.96	0.04	0.19 (0.04)	4.6×10^{-6}	0.53	0.88	0.72
rs61812508	1	147251772	A	G	0.05	0.95	-0.18 (0.04)	4.3×10^{-6}	0.16	0.77	0.24
rs7608236	2	180062867	A	G	0.29	0.71	-0.07 (0.02)	4.1×10^{-6}	0.15	0.23	0.64
rs116248825	3	26420135	A	C	0.04	0.96	-0.28 (0.06)	4.1×10^{-6}	0.09	0.74	0.87
rs112608607	5	102908739	T	C	0.97	0.03	0.22 (0.05)	3.8×10^{-6}	0.31	0.52	0.75
1:246689691:I	1	246689691	D	I	0.6	0.4	0.07 (0.02)	3.7×10^{-6}	0.01	0.77	0.47
rs9867656	3	30100084	A	G	0.34	0.66	-0.07 (0.01)	3.7×10^{-6}	0.99	0.14	0.86
rs1225927	6	7871037	T	G	0.75	0.25	0.07 (0.02)	3.5×10^{-6}	0.6	0.59	0.93
rs11583958	1	147291718	A	T	0.04	0.96	-0.18 (0.04)	3.3×10^{-6}	0.18	0.72	0.20
rs11711327	3	30101254	A	G	0.66	0.34	0.07 (0.01)	3.1×10^{-6}	0.99	0.14	0.86
rs114667268	2	12433490	T	C	0.01	0.99	-0.49 (0.10)	2.9×10^{-6}	0.46	0.66	0.23
rs113322056	5	102913288	A	G	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	0.33	0.83	0.74
rs146551307	5	102915236	T	C	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	0.33	0.83	0.75
rs702491	1	54194992	T	C	0.19	0.81	0.09 (0.02)	2.4×10^{-6}	0.71	0.17	0.10
1:3118674:D	1	3118674	D	I	0.12	0.88	0.11 (0.02)	1.8×10^{-6}	0.73	0.02	0.07
rs189819077	18	34933012	A	G	0.03	0.97	-0.20 (0.04)	1.8×10^{-6}	0.54	0.87	0.70
rs72811030	5	179729009	A	G	0.38	0.62	0.07 (0.02)	1.7×10^{-6}	0.71	0.36	0.31
rs6429657	1	14702354	A	G	0.96	0.04	-0.19 (0.04)	1.6×10^{-6}	0.14	0.93	0.43
rs62047859	16	76826391	A	T	0.03	0.97	0.21 (0.04)	1.0×10^{-6}	0.54	0.17	0.34

Abbreviations: A, adenine; BMI, body mass index; C, cytosine; Chr, chromosome; D, deletion; EA, effect allele; EAF, effect allele frequency; G, guanine; GWAS, genome-wide association study; OA, other allele; OAF, other allele frequency; RS, Rotterdam Study; SE, standard error; SNP, single-nucleotide polymorphism; T, thymine. Boldface indicates the top SNP.

[a]analyses are adjusted for age, sex, the first four genetic principal components, and technical variables of the digital measurement; [b]based on GRCh37/hg19; [c]P-value of the interaction term SNP*BMI, added as covariate in the linear regression; [d]P-value of the interaction term SNP*smoking, added as covariate in the linear regression.

Supplementary Table S6. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles[a] in the Rotterdam Study and their effects (β) and P-values tested in site-specific wrinkles (forehead wrinkles, crow's feet wrinkles, and in women upper lip wrinkles)

						Global facial wrinkles (N=3513)		Forehead wrinkles (N=3499)		Crow's feet wrinkles (N=3544)		Upper lip wrinkles women (N=1136)	
SNP	**Chr**	**Position[b]**	**EA**	**EAF**	**OAF**	**β (SE)**	**P-value**	**β (SE)**	**P-value**	**β (SE)**	**P-value**	**β (SE)**	**P-value**
rs10476781	**5**	**151763633**	**T**	**0.94**	**0.06**	**-0.21 (0.04)**	**9.5×10^{-8}**	**-0.26 (0.07)**	**1.40×10^{-4}**	**-0.13 (0.07)**	**5.2×10^{-2}**	**-0.22 (0.11)**	**5.4×10^{-2}**
rs185291539	10	84338421	A	0.98	0.02	0.41 (0.09)	4.8×10^{-6}	0.25 (0.15)	9.20×10^{-2}	0.25 (0.15)	9.0×10^{-2}	-0.03 (0.25)	0.91
5:102915644:D	5	102915644	D	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	-0.16 (0.07)	2.90×10^{-2}	-0.19 (0.07)	7.2×10^{-3}	-0.18 (0.11)	0.11
9:16847398:D	9	16847398	D	0.98	0.02	0.30 (0.07)	4.7×10^{-6}	0.29 (0.11)	9.40×10^{-3}	-0.08 (0.11)	0.49	0.01 (0.16)	0.94
rs72845240	17	61361539	C	0.04	0.96	-0.19 (0.04)	4.7×10^{-6}	-0.24 (0.07)	5.60×10^{-4}	-0.13 (0.07)	7.0×10^{-2}	-0.14 (0.11)	0.19
rs11577655	1	3119489	T	0.13	0.87	0.11 (0.02)	4.6×10^{-6}	0.09 (0.04)	3.10×10^{-2}	0.13 (0.04)	1.8×10^{-3}	0.26 (0.06)	3.9×10^{-5}
rs62077967	17	61253263	C	0.96	0.04	0.19 (0.04)	4.6×10^{-6}	0.24 (0.07)	5.30×10^{-4}	0.13 (0.07)	7.1×10^{-2}	0.14 (0.11)	0.18
rs61812508	1	147251772	A	0.05	0.95	-0.18 (0.04)	4.3×10^{-6}	-0.14 (0.07)	3.10×10^{-2}	-0.17 (0.07)	1.2×10^{-2}	-0.06 (0.10)	0.55
rs7608236	2	180062867	A	0.29	0.71	-0.07 (0.02)	4.1×10^{-6}	0.08 (0.03)	2.20×10^{-3}	-0.04 (0.03)	8.7×10^{-2}	-0.06 (0.04)	0.17
rs116248825	3	26420135	A	0.04	0.96	-0.28 (0.06)	4.1×10^{-6}	-0.14 (0.10)	0.17	-0.17 (0.10)	9.3×10^{-2}	-0.18 (0.15)	0.22
rs112608607	5	102908739	T	0.97	0.03	0.22 (0.05)	3.8×10^{-6}	0.23 (0.08)	3.60×10^{-3}	0.26 (0.08)	9.7×10^{-4}	0.21 (0.12)	9.1×10^{-2}
1:246689691:I	1	246689691	D	0.6	0.4	0.07 (0.02)	3.7×10^{-6}	0.08 (0.03)	4.10×10^{-3}	-0.001 (0.03)	0.98	0.04 (0.04)	0.28
rs9867656	3	30100084	A	0.34	0.66	-0.07 (0.01)	3.7×10^{-6}	0.04 (0.02)	7.50×10^{-2}	-0.09 (0.02)	2.0×10^{-4}	-0.06 (0.04)	0.11
rs1225927	6	7871037	T	0.75	0.25	0.07 (0.02)	3.5×10^{-6}	0.04 (0.03)	0.19	0.05 (0.03)	6.9×10^{-2}	0.07 (0.04)	0.12
rs11583958	1	147291718	A	0.04	0.96	-0.18 (0.04)	3.3×10^{-6}	-0.14 (0.07)	3.50×10^{-2}	-0.19 (0.07)	4.2×10^{-3}	-0.06 (0.10)	0.57
rs11711327	3	30101254	A	0.66	0.34	0.07 (0.01)	3.1×10^{-6}	0.04 (0.02)	8.70×10^{-2}	0.09 (0.02)	2.3×10^{-4}	0.06 (0.04)	0.11
rs114667268	2	12433490	T	0.01	0.99	-0.49 (0.10)	2.9×10^{-6}	-0.26 (0.15)	8.70×10^{-2}	-0.18 (0.15)	0.22	0.005 (0.22)	0.98
rs113322056	5	102913288	A	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	0.20 (0.07)	6.50×10^{-3}	0.23 (0.07)	1.8×10^{-3}	0.22 (0.11)	5.6×10^{-2}
rs146551307	5	102915236	T	0.96	0.04	0.20 (0.04)	2.9×10^{-6}	0.20 (0.07)	6.60×10^{-3}	0.23 (0.07)	1.7×10^{-3}	0.22 (0.11)	5.6×10^{-2}
rs702491	1	54194992	T	0.19	0.81	0.09 (0.02)	2.4×10^{-6}	0.08 (0.03)	1.10×10^{-2}	0.06 (0.03)	8.4×10^{-2}	0.08 (0.05)	0.11
1:3118674:D	1	3118674	D	0.12	0.88	0.11 (0.02)	1.8×10^{-6}	0.09 (0.04)	2.00×10^{-2}	0.13 (0.04)	1.5×10^{-3}	0.25 (0.06)	5.5×10^{-5}
rs189819077	18	34933012	A	0.03	0.97	-0.20 (0.04)	1.8×10^{-6}	-0.29 (0.07)	4.50×10^{-5}	-0.18 (0.07)	1.3×10^{-2}	0.07 (0.12)	0.54

Supplementary Table S6. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles[a] in the Rotterdam Study and their effects (β) and P-values tested in site-specific wrinkles (forehead wrinkles, crow's feet wrinkles, and in women upper lip wrinkles) (continued)

						Global facial wrinkles (N=3513)		Forehead wrinkles (N=3499)		Crow's feet wrinkles (N=3544)		Upper lip wrinkles women (N=1136)	
SNP	**Chr**	**Position[b]**	**EA**	**EAF**	**OAF**	**β (SE)**	**P-value**	**β (SE)**	**P-value**	**β (SE)**	**P-value**	**β (SE)**	**P-value**
rs72811030	5	179729009	A	0.38	0.62	0.07 (0.02)	1.7×10^{-6}	0.06 (0.03)	2.20 ×10^{-2}	0.01 (0.03)	0.60	0.09 (0.04)	2.7×10^{-2}
rs6429657	1	14702354	A	0.96	0.04	-0.19 (0.04)	1.6×10^{-6}	-0.10 (0.07)	0.14	-0.06 (0.07)	0.39	-0.002 (0.11)	0.98
rs62047859	16	76826391	A	0.03	0.97	0.21 (0.04)	1.0×10^{-6}	0.24 (0.07)	1.20 ×10^{-3}	0.15 (0.07)	4.5×10^{-2}	0.18 (0.11)	9.9×10^{-2}

Abbreviations: A, adenine; C, cytosine; Chr, chromosome; D, deletion; EA, effect allele; EAF, effect allele frequency; G, guanine; GWAS, genome-wide association study; OA, other allele; OAF, other allele frequency; SE, standard error; SNP, single-nucleotide polymorphism; T, thymine. Boldface indicates the top SNP.

[a]analyses are adjusted for age, sex, the first four genetic principal components, and technical variables of the digital measurement; [b]based on GRCh37/hg19.

Supplementary Table S7. Previously suggested SNPs significantly associated with facial aging in other recent GWAS and their associations with global facial wrinkles[a] in the Rotterdam Study, the Leiden Longevity Study, and the meta-analysis of both cohorts

					RS (N=3513)				LLS (N=599)				Meta-analysis (RS & LLS, N=4112)			
SNP	Chr	Position[b]	Published P-value	Associated phenotype	EA	EAF	β (SE)	P-value	EA	EAF	β (SE)	P-value	EA	Dir	Z[c]	P-value
rs7616661[d]	3	5965543	4.8×10^{-8}	Photoaging	T	0.98	-0.05 (0.06)	0.37	T	0.98	-0.43 (0.26)	0.09	T	--	-1.47	0.19
rs6975107[d]	7	120380907	4.2×10^{-9}	Photoaging	n.a.	n.a.	n.a.	n.a.	T	0.002	-0.25 (1.57)	0.88	T	?-	-0.16	0.88
rs11863929[d]	16	88304433	1.8×10^{-8}	Photoaging	n.a.	n.a.	n.a.	n.a.	G	0.01	-0.17 (0.58)	0.77	C	?+	0.29	0.77
rs322458[e]	3	120585315	1.5×10^{-8}	Photoaging	T	0.37	-0.01 (0.01)	0.34	C	0.63	0.00 (0.07)	0.97	T	--	-0.89	0.38
rs12203592[f]	6	396321	1.9×10^{-27}	PS	T	0.10	-0.04 (0.02)	0.10	C	0.92	-0.27 (0.12)	0.03	T	-+	-0.67	0.50
rs62543565[f]	9	16901067	1.5×10^{-7}	PS	A	0.60	-0.01 (0.01)	0.70	C	0.41	-0.01 (0.07)	0.94	A	-+	-0.33	0.74
rs35063026[f]	16	89736157	9.4×10^{-15}	PS	T	0.07	-0.01 (0.03)	0.79	C	0.92	-0.10 (0.13)	0.45	T	-+	0.05	0.96
rs6059655[f]	20	32665748	2.6×10^{-9}	PS	A	0.08	-0.00 (0.03)	0.95	A	0.10	0.02 (0.12)	0.86	A	-+	0.01	0.99
rs11876749[g]	18	3942902	1.7×10^{-8}	Sagging eyelids	T	0.52	0.01 (0.01)	0.61	T	0.51	-0.00 (0.07)	0.97	T	+-	0.46	0.65
rs185146[h]	5	33952106	4.1×10^{-9}	MT	T	0.98	-0.04 (0.05)	0.50	C	0.02	-0.44 (0.32)	0.17	T	-+	-0.11	0.92
rs12203592[h]	6	396321	8.8×10^{-13}	MT	T	0.10	-0.04 (0.02)	0.10	C	0.92	-0.27 (0.12)	0.03	T	-+	-0.67	0.50
rs4268748[h]	16	90026512	1.2×10^{-15}	MT	T	0.73	0.00 (0.02)	0.83	T	0.75	-0.12 (0.08)	0.14	T	+-	-0.36	0.72
rs1805007[h]	16	89986117	1.2×10^{-10}	MT	T	0.07	0.00 (0.03)	0.89	C	0.93	-0.19 (0.13)	0.16	T	++	0.66	0.51
rs1805008[h]	16	89986144	1.1×10^{-5}	MT	T	0.10	-0.02 (0.02)	0.49	C	0.91	-0.04 (0.12)	0.71	T	-+	-0.50	0.62

Abbreviations: A, adenine; C, cytosine; Chr, chromosome; Dir, direction of the effects; EA, effect allele; EAF, effect allele frequency; G, guanine; GWAS, genome-wide association study; LLS, Leiden Longevity Study; MT, microtopography score; PS, pigmented spots; RS, Rotterdam Study; SE, standard error; SNP, single-nucleotide polymorphism; T, thymine.

[a]analyses are adjusted for age, sex, and the first four genetic principal components and in addition, for the RS, for technical variables of the digital measurement; [b]based on GRCh37/hg19; [c]weighted Z-score; [d]SNPs found by Chang et al.; [e]SNP found by Le Clerc et al.; [f]SNPs found by Jacobs et al. for pigmented spots; [g]SNP found by Jacobs et al. for sagging eyelids; [h]SNPs found bij Law et al. in a genome-wide meta-analysis for microtopography score of the back of the hand.

Supplementary Table S8. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles in the Rotterdam Study, in a univariate analysis (excluding age and sex)[a]

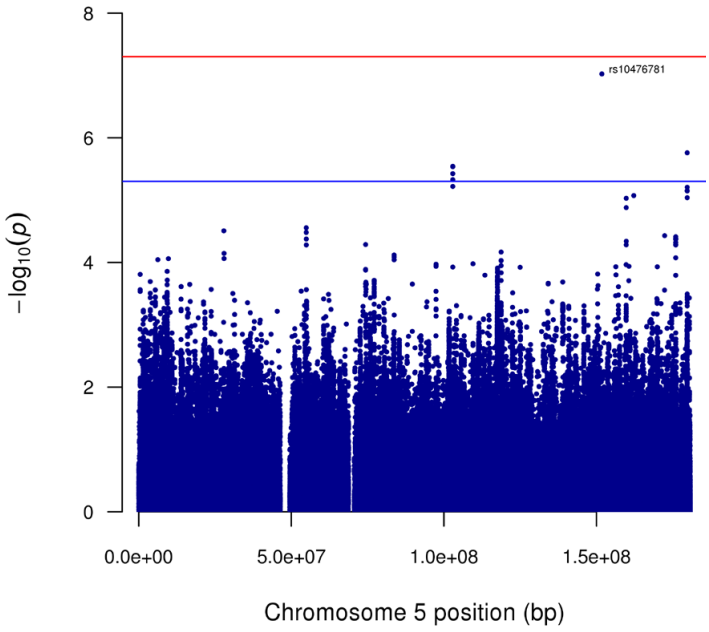
			RS (N=3513)					
SNP	**Chr**	**Position[b]**	**EA**	**OA**	**EAF**	**OAF**	**β (SE)**	**P-value**
rs1857883	3	30052399	A	G	0.54	0.46	0.07 (0.01)	1.2×10^{-7}
rs10476781	**5**	**151763633**	**T**	**C**	**0.94**	**0.06**	**-0.22 (0.04)**	**1.3×10^{-7}**
3:30071057:I	3	30071057	D	I	0.46	0.54	-0.08 (0.01)	1.9×10^{-7}
rs34836863	3	30058003	T	C	0.54	0.46	0.07 (0.01)	2.9×10^{-7}
rs9867656	3	30100084	A	G	0.34	0.66	-0.08 (0.02)	3.4×10^{-7}
rs11711327	3	30101254	A	G	0.66	0.34	0.08 (0.02)	3.6×10^{-7}
rs11129380	3	30072352	T	C	0.45	0.55	-0.07 (0.01)	5.6×10^{-7}
rs11925126	3	30072451	T	C	0.45	0.55	-0.07 (0.01)	5.6×10^{-7}
rs961878	3	30073620	A	G	0.55	0.45	0.07 (0.01)	5.6×10^{-7}
rs1506298	3	30074228	C	G	0.55	0.45	0.07 (0.01)	5.7×10^{-7}
3:30072552:I	3	30072552	D	I	0.45	0.55	-0.07 (0.01)	5.8×10^{-7}
rs6549970	3	30064134	T	C	0.55	0.45	0.07 (0.01)	6.2×10^{-7}
rs1506287	3	30068387	T	G	0.45	0.55	-0.07 (0.01)	6.2×10^{-7}
rs9847686	3	30064795	T	C	0.45	0.55	-0.07 (0.01)	6.6×10^{-7}
rs2371911	3	30065907	A	T	0.55	0.45	0.07 (0.01)	6.6×10^{-7}
rs6795173	3	30055039	A	G	0.57	0.43	-0.07 (0.01)	6.9×10^{-7}
rs61812508	1	147251772	A	G	0.04	0.96	-0.20 (0.04)	7.3×10^{-7}
rs1506285	3	30068158	A	G	0.55	0.45	0.07 (0.01)	7.4×10^{-7}
rs11583958	1	147291718	A	T	0.04	0.96	-0.20 (0.04)	7.8×10^{-7}
rs1506290	3	30069645	T	C	0.55	0.45	0.07 (0.01)	8.0×10^{-7}
3:30058483:D	3	30058483	D	I	0.52	0.48	0.07 (0.01)	8.6×10^{-7}
rs7426945	3	30071380	A	G	0.45	0.55	-0.07 (0.01)	8.6×10^{-7}
rs141920505	3	30095549	A	C	0.35	0.65	-0.07 (0.02)	8.6×10^{-7}
rs72811030	5	179729009	A	G	0.38	0.62	0.08 (0.02)	9.9×10^{-7}
rs61809935	1	147272111	T	C	0.95	0.05	0.20 (0.04)	1.0×10^{-6}
rs7649490	3	30111359	A	G	0.65	0.35	0.07 (0.01)	1.5×10^{-6}
rs6804839	3	30063702	A	G	0.46	0.54	-0.07 (0.01)	1.7×10^{-6}
rs77548552	9	20970280	A	C	0.97	0.03	0.22 (0.05)	1.7×10^{-6}
rs4626055	3	30061866	T	C	0.46	0.54	-0.07 (0.01)	2.2×10^{-6}
rs62047859	16	76826391	A	T	0.03	0.97	0.21 (0.05)	2.4×10^{-6}
rs4426642	3	30074874	A	G	0.44	0.56	-0.07 (0.01)	2.8×10^{-6}
rs55848714	5	179734528	A	C	0.6	0.4	-0.07 (0.01)	3.0×10^{-6}
rs184810693	7	45992257	T	C	0.01	0.99	0.76 (0.16)	3.0×10^{-6}
rs62404922	5	179731466	A	C	0.4	0.6	0.07 (0.01)	3.6×10^{-6}
rs729652	5	179731695	T	C	0.4	0.6	0.07 (0.01)	3.6×10^{-6}
rs62404925	5	179732797	T	C	0.4	0.6	0.07 (0.01)	3.6×10^{-6}
rs12596502	16	83872697	T	G	0.2	0.8	-0.08 (0.02)	3.7×10^{-6}
rs138860040	11	31511241	T	C	0.01	0.99	-0.47 (0.10)	3.8×10^{-6}

Supplementary Table S8. Top SNPs (P-values <5×10^{-6}) of the GWAS for global facial wrinkles in the Rotterdam Study, in a univariate analysis (excluding age and sex)[a] (continued)

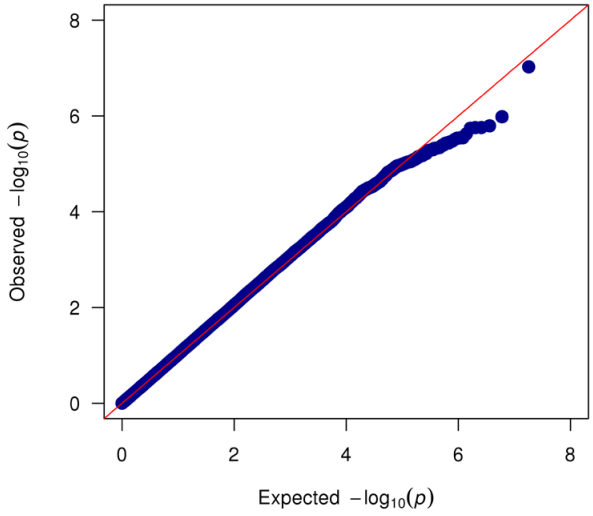
			RS (N=3513)					
SNP	**Chr**	**Position[b]**	**EA**	**OA**	**EAF**	**OAF**	**β (SE)**	**P-value**
rs189819077	18	34933012	A	G	0.03	0.97	-0.20 (0.04)	3.9×10^{-6}
rs76007816	9	20766718	A	G	0.98	0.02	0.25 (0.05)	4.2×10^{-6}
6:105164470:D	6	105164470	D	I	0.38	0.62	-0.07 (0.02)	4.3×10^{-6}
rs12494646	3	30076692	A	G	0.56	0.44	0.07 (0.01)	4.4×10^{-6}
rs2888213	3	30077137	A	G	0.44	0.56	-0.07 (0.01)	4.4×10^{-6}
rs78440239	5	150293381	T	C	0.02	0.98	-0.25 (0.06)	4.5×10^{-6}
rs9837298	3	30066066	A	G	0.52	0.48	0.07 (0.01)	4.6×10^{-6}
5:162220036	5	162220036	A	G	0.01	0.99	-1.20 (0.26)	4.8×10^{-6}
rs34280244	3	30098568	T	C	0.62	0.38	0.07 (0.01)	4.9×10^{-6}
rs118178650	9	20776109	T	C	0.02	0.98	-0.24 (0.05)	4.9×10^{-6}
rs12599838	16	83871039	A	C	0.2	0.8	-0.08 (0.02)	4.9×10^{-6}
rs6549971	3	30064282	A	G	0.48	0.52	-0.06 (0.01)	5.0×10^{-6}
rs62404924	5	179732771	A	G	0.6	0.4	-0.07 (0.01)	5.0×10^{-6}

Abbreviations: A, adenine; C, cytosine; Chr, chromosome; EA, effect allele; EAF, effect allele frequency; G, guanine; GWAS, genome-wide association study; RS, Rotterdam Study; SE, standard error; SNP, single-nucleotide polymorphism; T, thymine. Boldface indicates the top SNP.

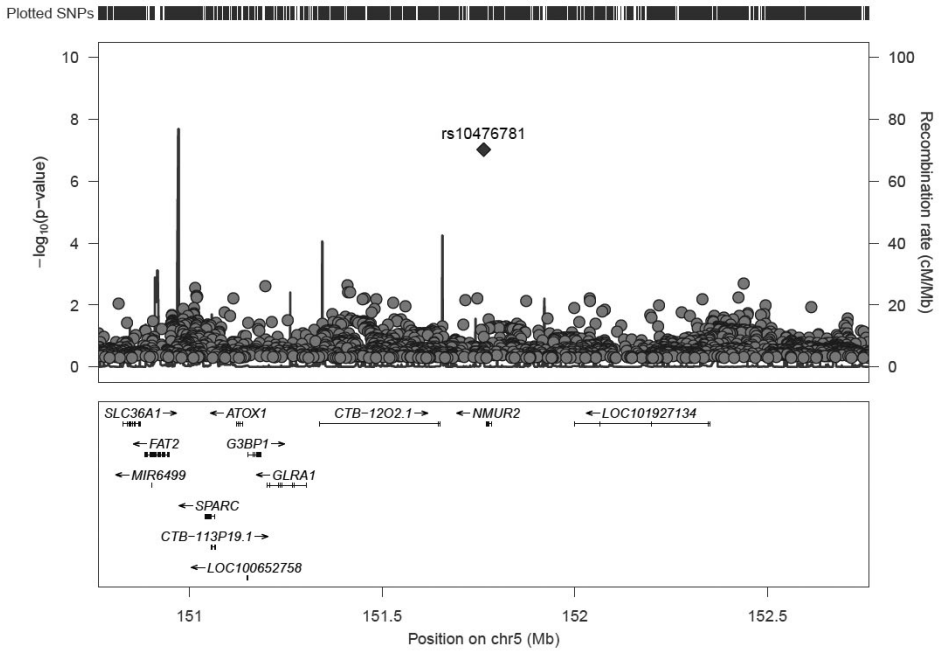
[a]analyses are adjusted for technical variables of the digital measurement and the first four genetic principal components; [b]based on GRCh37/hg19.



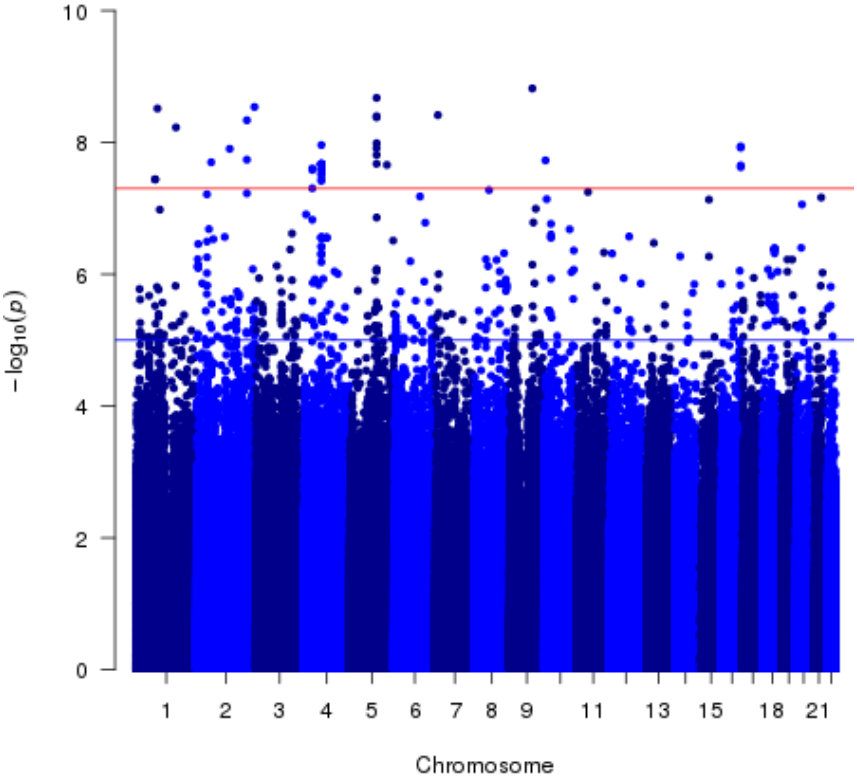
Supplementary Figure S1. Manhattan plot of the genome-wide associations for wrinkle area on chromosome 5 in the discovery cohort (Rotterdam Study, N=3513). All SNPs are represented by dots; x-axis shows the base pairs on chromosome 5; y-axis shows negative $\log_{10}$-transformed P-values.



Supplementary Figure S2. Quantile-quantile (QQ) plot of the expected (x-axis) vs. observed (y-axis) P-values of the genome-wide associations for wrinkle area in the discovery cohort (Rotterdam Study, N=3513). The higher observed than expected P-values are due to the lack of power in our study.



Supplementary Figure S3. LocusZoom[25] plot showing the region on chromosome 5 around the top suggestive SNP rs10476781 associated with wrinkle area in the discovery cohort (Rotterdam Study, N=3513). There is no LD between rs10476781 and other SNPs shown in this region.



Supplementary Figure S4. Manhattan plot of the genome-wide associations for wrinkle area in the meta-analysis of the two cohorts (Rotterdam Study, N=3513 & Leiden Longevity Study, **N=599).** All SNPs are represented by dots and displayed per chromosome (x-axis); y-axis shows negative $\log_{10}$-transformed P-values. There are multiple significant associations of SNPs that were only present in the LLS. Because of the small sample size in the LLS, these are likely to be false positives and therefore not reported as significant hits.

REFERENCES

1. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol*. 2015;30(8):661-708.
2. Schoenmaker M, de Craen AJ, de Meijer PH, Beekman M, Blauw GJ, Slagboom PE, et al. Evidence of genetic enrichment for exceptional survival using a family approach: the Leiden Longevity Study. *Eur J Hum Genet*. 2006;14(1):79-84.
3. Westendorp RG, van Heemst D, Rozing MP, Frolich M, Mooijaart SP, Blauw GJ, et al. Nonagenarian siblings and their offspring display lower risk of mortality and morbidity than sporadic nonagenarians: The Leiden Longevity Study. *J Am Geriatr Soc*. 2009;57(9):1634-7.
4. Hamer MA, Jacobs LC, Lall JS, Wollstein A, Hollestein LM, Rae AR, et al. Validation of image analysis techniques to measure skin aging features from facial photographs. *Skin Res Technol*. 2015;21(4):392-402.
5. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M, et al. Lifestyle and physiological factors associated with facial wrinkling in men and women. *J Invest Dermatol*. 2017.
6. Gunn DA, Rexbye H, Griffiths CE, Murray PG, Fereday A, Catt SD, et al. Why some women look young for their age. *PLoS One*. 2009;4(12):e8021.
7. Genomes Project C, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491(7422):56-65.
8. Deelen J, Beekman M, Uh HW, Broer L, Ayers KL, Tan Q, et al. Genome-wide association meta-analysis of human longevity identifies a novel locus conferring survival beyond 90 years of age. *Hum Mol Genet*. 2014;23(16):4420-32.
9. Uh HW, Beekman M, Meulenbelt I, Houwing-Duistermaat JJ. Genotype-Based Score Test for Association Testing in Families. *Stat Biosci*. 2015;7(2):394-416.
10. Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. 2010;11:134.
11. Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Magi R, et al. Quality control and conduct of genome-wide association meta-analyses. *Nat Protoc*. 2014;9(5):1192-212.
12. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26(17):2190-1.
13. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88(1):76-82.
14. Benzon CR, Johnson SB, McCue DL, Li D, Green TA, Hommel JD. Neuromedin U receptor 2 knockdown in the paraventricular nucleus modifies behavioral responses to obesogenic high-fat food and leads to increased body weight. *Neuroscience*. 2014;258:270-9.
15. Hainerova I, Torekov SS, Ek J, Finkova M, Borch-Johnsen K, Jorgensen T, et al. Association between neuromedin U gene variants and overweight and obesity. *J Clin Endocrinol Metab*. 2006;91(12):5057-63.
16. Ernster VL, Grady D, Miike R, Black D, Selby J, Kerlikowske K. Facial wrinkling in men and women, by smoking status. *Am J Public Health*. 1995;85(1):78-82.
17. Chang AL, Atzmon G, Bergman A, Brugmann S, Atwood SX, Chang HY, et al. Identification of genes promoting skin youthfulness by genome-wide association study. *J Invest Dermatol*. 2014;134(3):651-7.
18. Le Clerc S, Taing L, Ezzedine K, Latreille J, Delaneau O, Labib T, 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*. 2013;133(4):929-35.

19. Jacobs LC, Hamer MA, Gunn DA, Deelen J, Lall JS, van Heemst D, et al. A Genome-Wide Association Study Identifies the Skin Color Genes IRF4, MC1R, ASIP, and BNC2 Influencing Facial Pigmented Spots. *J Invest Dermatol*. 2015;135(7):1735-42.
20. Jacobs LC, Liu F, Bleyen I, Gunn DA, Hofman A, Klaver CC, et al. Intrinsic and extrinsic risk factors for sagging eyelids. *JAMA Dermatol*. 2014;150(8):836-43.
21. Law MH, Medland SE, Zhu G, Yazar S, Vinuela A, Wallace L, et al. Genome-Wide Association Shows that Pigmentation Genes Play a Role in Skin Aging. *J Invest Dermatol*. 2017;137(9):1887-94.
22. Peng B, Yu RK, Dehoff KL, Amos CI. Normalizing a large number of quantitative traits using empirical normal quantile transformation. *BMC Proc*. 2007;1 Suppl 1:S156.
23. Feng S, Wang S, Chen CC, Lan L. GWAPower: a statistical power calculation software for genome-wide association studies with quantitative traits. *BMC Genet*. 2011;12:12.
24. Larnier C, Ortonne JP, Venot A, Faivre B, Beani JC, Thomas P, et al. Evaluation of cutaneous photodamage using a photographic scale. *Br J Dermatol*. 1994;130(2):167-73.
25. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26(18):2336-7.