Facial Wrinkles in Europeans: a Genome-Wide Association Study

Merel A. Hamer[1], Luba M. Pardo[1] ,Leonie C. Jacobs[1], Joris Deelen[2,3], André G. Uitterlinden[4,5], Eline Slagboom[2], Diana van Heemst[6], Hae-Won Uh[2], Marian Beekman[2], Manfred Kayser[7], Fan Liu[7,8,9], David A. Gunn[10], Tamar Nijsten[1]

Affiliations:
[1]Department of Dermatology, [4]Department of Epidemiology, [5]Department of Internal Medicine, [7]Department of Genetic Identification; all from Erasmus MC University Medical Center Rotterdam, Rotterdam, The Netherlands
[2]Department of Molecular Epidemiology, [6]Department of Internal Medicine, section Gerontology and Geriatrics; both from Leiden University Medical Center, Leiden, The Netherlands
[3]Max Planck Institute for Biology of Ageing, Cologne, Germany
[8]Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, China
[9]University of Chinese Academy of Sciences, Beijing, China
[10]Unilever R&D, Colworth Science Park, Sharnbrook, Bedfordshire, MK44 1LQ, United Kingdom

Short title: GWAS for facial wrinkles

Abbreviations:

GWAS	: Genome-wide association study
LD	: Linkage disequilibrium
LLS	: Leiden Longevity Study
PA	: Perceived age
RS	: Rotterdam Study
SNP	: Single nucleotide polymorphism

Corresponding author:
Tamar Nijsten
Erasmus MC – Dermatology department
Burgemeester s'Jacobplein 51
3015 CA Rotterdam
Telephone: +31 10 703 45 80
Fax: +31 10 703 38 22
Email: t.nijsten@erasmusmc.nl

TO THE EDITOR,

Wrinkles are among the most notable components of skin aging and are influenced by many different risk factors (Hamer et al., 2017). Although wrinkle variation has been shown to be a heritable trait, (55%, (Gunn et al., 2009)), specific gene variants for wrinkles have not yet been identified. Previous studies have identified the *MC1R* gene influencing skin photoaging and pigmented spots (Elfakir et al., 2010, Jacobs et al., 2015, Liu et al., 2016, Suppa et al., 2011), but its role in wrinkling is not clear. In this study, we performed the largest GWAS for global facial wrinkles available to date in 3,513 participants from the Rotterdam Study (RS) using a digital wrinkle measure (Hamer et al., 2017) 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 (Hofman et al., 2015). The current study includes 3,513 north-western European participants, for whom standardized facial photographs and quality-controlled genotype data were available. The LLS is a family-based study (Westendorp et al., 2009), including 599 participants for the current 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 (Gunn et al., 2009). In the RS, DNA from whole blood was extracted following standard protocols and quality controls were applied on markers and individuals (Hofman et al., 2015). Imputations were performed with 1000Genomes (GIANT Phase I version 3) as the reference panel (Genomes Project et al., 2012). In total 30,072,738 markers were genotyped/imputed. After quality controls, 9,009,554 autosomal SNPs

were available. In the LLS, imputation was performed similarly and association testing was conducted using QT-assoc (Uh et al., 2015). The RS served as discovery dataset. We performed linear regression using an additive model (SNP dosage data, (Aulchenko et al., 2010)) 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 (Hamer et al., 2017). 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 (Jacobs et al., 2015a). 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, BMI and smoking; 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, the majority were women (n=2,045, 58.2%) and the median age was 66.2 (range 51-98; men 66.5, range 51-96; women 66.0 range 51-98) years. Men showed a higher average wrinkle area (median facial wrinkle area 4.4%, 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% 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 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 (SE 0.04).

Estimating pairwise LD between all SNPs with suggestive associations (25 SNPs, Table 1) resulted in 11 independent loci ($r^2 \leq 0.5$). Of note, there was no LD between rs10476781 and other suggestive SNPs in our dataset ($r^2 \leq 0.5$, Supplementary Table S2, 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, while 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 S7 and Supplementary Figure S4. Additional genome-wide meta-analysis of the RS and LLS did not reveal any new findings (Supplementary Material, including Table S7).

Because of known sex differences in facial wrinkling (Hamer et al., 2017), 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 S3).

This is the largest GWAS of global facial wrinkling conducted thus far, in which we found that the rs10476781 SNP was a suggestive hit for global facial wrinkling in the RS (3,513 north-

western Europeans) and a significant genome-wide hit in a meta-analysis of the RS and LLS cohorts together (n=4,122). However, we cannot exclude that this may be a false positive finding since the imputation score in the RS was moderate, and the SNP has a very low frequency in the general population (MAF<0.01, and thus was not included in the latest release of 1000Genomes).The latter likely explains the moderate imputation quality as rare variants are more difficult to impute. However, it has a higher frequency in Dutch populations (GoNL, a Dutch-specific reference dataset; 2% MAF, with a low quality though), 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

The *MC1R* gene influences skin aging (Elfakir et al., 2010, Law et al., 2017, Liu et al., 2016, Suppa et al., 2011). However, we did not find any significant association between *MC1R* variants and wrinkles, which suggests these variants are not influencing facial wrinkle variation as measured in the RS cohort, but instead other skin aging phenotypes, e.g. pigmented age spots (Jacobs et al., 2015). Furthermore, we did not replicate SNPs previously reported as associated with skin aging, bar 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 due to the small sample sizes (Ioannidis, 2003), or due to phenotypic heterogeneity in photoaging versus wrinkling in our study. Also, genetic heterogeneity could play a role.

We cannot exclude that other SNPs may be associated with wrinkling, since the heritability was 42% in the RS (P-value 4.4×10^{-8}, 95%CI 28%–61%, (Yang et al., 2010)). Most probably the effects of each influencing SNP are too small to be detected with a sample size as used in this study since we had a 77% power to detect SNPs with moderate effects (Supplementary Results).

This highlights the importance of increasing sample sizes for future GWAS. 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 (Hamer et al., 2015)), hence we believe 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 north-western European cohorts. This intergenic SNP (628 KB downstream of the Neuromedin U Receptor gene) is an interesting candidate but needs further validation.

CONFLICT OF INTEREST

Although no products were tested, it is possible this manuscript could promote products that reduce the appearance of wrinkles, which could lead to financial gain for Unilever.

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.

FUNDING

This study is funded by Unilever. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University Rotterdam; Netherlands Organization for the Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. Author MAH is supported by Unilever and author DAG is a Unilever employee. Bar the author DAG, the funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The LLS has received funding from the European Union's Seventh Framework Programme (FP7/2007-2011) under grant agreement no 259679. This study was supported by a grant from the Innovation-Oriented Research Program on Genomics (SenterNovem IGE05007), the Centre for Medical Systems Biology, and the Netherlands Consortium for Healthy Ageing (grants 05040202 and 050060810), all in the framework of the Netherlands Genomics Initiative, Netherlands Organization for Scientific Research (NWO), Unilever Colworth, and by BBMRINL, a Research Infrastructure financed by the Dutch government (NWO 184.021.007). JD is financially supported by the Alexander von Humboldt Foundation.

REFERENCES

Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. BMC Bioinformatics 2010;11:134.

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.

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.

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.

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.

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.

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.

Ioannidis JP. Genetic associations: false or true? Trends Mol Med 2003;9(4):135-8.

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.

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.

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.

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.

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.

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.

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.

Table 1. Top SNP (P-values $<5\times10^{-6}$) of the GWAS for global facial wrinkles in the Rotterdam Study (RS, discovery cohort) and Leiden Longevity Study (LLS, replication cohort) and a meta-analysis of these 2 cohorts.

			Discovery cohort (RS, n=3,513)						Replication cohort (LLS, n=599)				Meta-analysis (RS & LLS, n=4,112)						
SNP	**Chr**	**Position***	**EA**	**OA**	**EAF**	**OAF**	**Beta (SE)**	**P-value**	**EA**	**EAF**	**Beta (SE)**	**P-value**	**EA**	**Dir**	**Z****	**P-value**	**I^2**	**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	147023 54	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	541949 92	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	147251 772	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	147291 718	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	246689 691	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	124334 90	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	180062 867	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	264201 35	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	301000 84	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	301012 54	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	102908 739	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

Analyses are adjusted for age, sex and the first four genetic principal components; additionally, for the RS also for technical variables of the digital measurement. *Based on GRCh37/hg19; **weighted Z-score. Abbreviations: SNP, single nucleotide polymorphism; Chr, chromosome; EA, effect allele; OA, other allele; EAF, effect allele frequency; OAF, other allele frequency; SE, standard error; Dir, direction of the effects; I^2, heterogeneity I^2; Het P-value, heterogeneity P-value.

Figure 1. Manhattan plot of the GWAS associations for wrinkle area in the discovery cohort (Rotterdam Study, n=3,513). All SNPs are represented by dots and displayed per chromosome (X-axis); Y-axis shows negative log10-transformed P-values.