Facial Wrinkles in Europeans: A Genome-Wide Association Study

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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 as 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 genome-wide association study (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 Materials online. The RS is an ongoing Dutch prospective population-based cohort study of 14,926 participants aged 45 years or older (Hofman et al., 2015). This study includes 3,513 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, which are 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 (Westendorp et al., 2009) that includes 599 participants in this study. In the RS, wrinkle area was digitally quantified as wrinkle area percentage of the face using semiautomated image analysis of high-resolution facial photographs. For wrinkle grading in the LLS, a 9-point photonumeric scale was used (Gunn et al., 2009). 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 (Hofman et al., 2015). Imputations were performed with 1000 Genomes (GIANT phase I version 3) as the reference panel (1000 Genomes Project Consortium et al., 2012). 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 (Uh et al., 2015). The RS served as the 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., 2015). We selected all SNPs with *P*-values less than 5×10^{-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 (see Supplementary Materials).

In the RS, most participants were women (n = 2,045, 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 = 2.9–6.2) than women (3.5%, interquartile range = 2.1–5.5). In the LLS, the mean age was 63.1 years, and 53.8% of participants were women (see Supplementary Table S1 online). The GWAS of global facial wrinkle area in the RS yielded 25 suggestive hits (*P*-values $< 5 \times 10^{-6}$) (Table 1), but none of them were genome-wide significant (Figure 1, and see Supplementary Figures S1 and S2 online). The strongest signal was found for an intergenic SNP (rs10476781; *P*-value $= 9.5 \times 10^{-8}$) on chromosome 5 between the *NMUR2* and *CTB-1202.1* (i.e., *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

Abbreviations: GWAS, genome-wide association study; LD, linkage disequilibrium; LLS, Leiden Longevity Study; RS, Rotterdam Study; SNP, single nucleotide polymorphism

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Table 1. Top SNP (*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[1]

			Discovery Cohort: RS (n = 3,513)						Replication Cohort: LLS (n = 599)				Meta-analysis (RS and LLS, n = 4,112)						
SNP	Chr	Position[2]	EA	OA	EAF	OAF	β (SE)	*P*-Value	EA	EAF	B (SE)	P-Value	EA	Dir	*Z*[3]	*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	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)**	$\mathbf{9.5 \times 10^{-8}}$	**T**	**0.94**	**−0.33 (0.19)**	**0.08**	**T**	−−	**−5.60**	$\mathbf{2.2 \times 10^{-8}}$	**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.

[1]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.

[2]Based on GRCh37/hg19.

[3]Weighted *Z*-score.

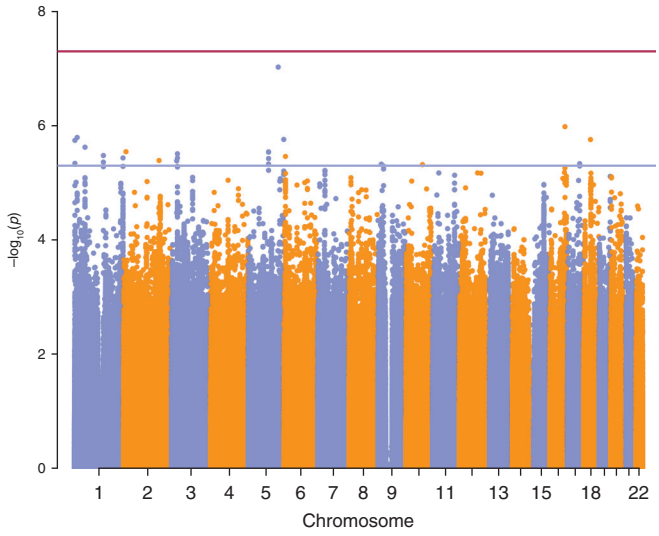

Figure 1. Manhattan plot of the genome-wide association studies for wrinkle area in the discovery cohort (Rotterdam Study, n = 3,513). All single-nucleotide polymorphisms are represented by dots and displayed per chromosome (x-axis); y-axis shows negative $\log_{10}$-transfomred *P*-values.

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 ($r^2 \leq 0.5$). There was no LD between rs10476781 and other suggestive SNPs in our dataset ($r^2 \leq 0.5$) (see Supplementary Table S2 and Supplementary Figure S3 online). 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 $\leq 5 \times 10^{-6}$) from the genome-wide meta-analysis of the two cohorts are presented in Supplementary Table S3 and Supplementary Figure S4 online. Additional genome-wide meta-analysis of the RS and LLS did not yield any new findings (see Supplementary Materials and Supplementary Table S3).

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 (see Supplementary Table S4 online).

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 (3,513 northwestern 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 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; Boomsma et al., 2014), 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 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 (Jacobs et al., 2015). 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 (Ioannidis, 2003) or because of 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, because the heritability was 42% in the RS (*P*-value = 4.4×10^{-8}, 95% CI = 28%–61%) (Yang et al., 2010). 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 (see Supplementary Materials, and Supplementary Tables S5–S8 online). 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 ρ = 0.8–0.9) (Hamer et al., 2015); 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 *NMUR2* gene) is an interesting candidate but needs further validation.

CONFLICT OF INTEREST

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

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.12.037.

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