Intrinsic and Extrinsic Risk Factors for Sagging Eyelids

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IMPORTANCE Sagging eyelids, or dermatochalasis, are a frequent concern in older adults. It is considered a feature of skin aging, but risk factors other than aging are largely unknown.

OBJECTIVE To study nongenetic and genetic risk factors for sagging eyelids.

DESIGN Upper eyelid sagging was graded in 4 categories of severity using digital photographs. Dermatochalasis was defined as the eyelid hanging over the eyelashes. Age, sex, skin color, tanning ability, hormonal status in women, current smoking, body mass index, and sun protection behavior were analyzed in a multivariable multinomial logistic regression model. Genetic predisposition was assessed using heritability analysis and a genome-wide association study.

SETTING AND PARTICIPANTS The study was performed in 2 independent population-based cohorts. The Rotterdam Study included older adults from one district in Rotterdam, the Netherlands, and the UK Adult Twin Registry (TwinsUK) included twins from all over the United Kingdom. Participants were 5578 unrelated Dutch Europeans (mean age, 67.1 years; 44.0% male) from the Rotterdam Study and 2186 twins (mean age, 53.1 years; 10.4% male) from the TwinsUK.

MAIN OUTCOMES AND MEASURES Sagging eyelid severity levels, ranging from 1 (normal control) to 4 (severe sagging).

RESULTS Among 5578 individuals from the Rotterdam Study, 17.8% showed dermatochalasis (moderate and severe sagging eyelids). Significant and independent risk factors for sagging eyelids included age, male sex, lighter skin color, and higher body mass index. In addition, current smoking was borderline significantly associated. Heritability of sagging eyelids was estimated to be 61% among 1052 twin pairs from the TwinsUK (15.6% showed dermatochalasis). A meta-analysis of genome-wide association study results from 5578 Rotterdam Study and 1053 TwinsUK participants showed a genome-wide significant recessive protective effect of the C allele of rs11876749 (P = 1.7 × 10⁻8). This variant is located close to TGIF1 (an inducer of transforming growth factor β), which is a known gene associated with skin aging.

CONCLUSIONS AND RELEVANCE This is the first observational study to date demonstrating that other risk factors (male sex, genetic variants, lighter skin color, high body mass index, and possibly current smoking) in addition to aging are involved in the origin of sagging eyelids.
Excess eyelid skin is known as dermatochalasis and is typically seen in middle-aged or older adults. Sagging eyelids are usually a cosmetic concern, although they can cause visual field loss, ocular or eyelid irritation, and headaches due to forced brow elevation to increase the visual field.

Sagging eyelids are considered a feature of the aging process of skin. Histologic examination of sagging eyelid skin demonstrates a loss of elastic fibers and a disruption of the collagen network, which are comparable to the changes observed in aged facial skin. The risk factors for sagging eyelids probably overlap with those for skin sagging in general (e.g., sagging of the cheeks or bags under the eyes), but whether the risk profile overlaps with that of the much wider investigated skin wrinkling is unclear. Skin sagging is mostly recognized as a result of intrinsic skin aging, but extrinsic aging could also have a role. To date, no observational studies have examined the risk factors for sagging eyelids. Expert opinion suggests that intrinsic factors, such as racial/ethnic background and sex, are not associated with dermatochalasis, whereas extrinsic factors (including sun exposure, smoking, and poor nutrition) increase the risk of sagging eyelids.

It has also been observed that certain families are predisposed to sagging eyelids. Two genodermatoses are associated with dermatochalasis. One is cutis laxa, a disease caused by mutations in the \textit{elastin} or \textit{fibulin} genes and characterized by skin sagging, including sagging eyelids, in affected individuals at a young age. The other is the connective tissue disease Ehlers-Danlos syndrome classic type, which includes skin hyperextensibility and sagging eyelids caused by genetic defects in collagens I and V. In addition, a recent genome-wide association study (GWAS) on skin photoaging (including skin sagging) found a putative role for the \textit{STXBPSL} gene, which suggests that genetic variation might be important and could partly explain the cause of sagging eyelids.

Herein, we graded eyelid sagging from digital photographs of 5578 Dutch Europeans from the Rotterdam Study (RS) and 2186 twins from the United Kingdom Adult Twin Registry (TwinsUK). We then investigated intrinsic (including genetic) and extrinsic risk factors for dermatochalasis by epidemiological and genetic association studies.

## Methods

### Study Population

#### Rotterdam Study

The medical ethics committee of the Erasmus Medical Center, Rotterdam, the Netherlands, approved the RS protocol, and all participants provided written informed consent. The RS is a population-based prospective study of 14,926 adults 45 years or older living in the same suburb of Rotterdam. After performing all phenotypic and genotypic quality controls, the present study included 5578 participants of North European ancestry for whom eye photographs and genotype data were available after quality controls. High-resolution eye photographs were obtained as described in a previous study. Photograpy was standardized by placing the chin in an adjustable chin rest and pushing the forehead against a horizontal bar, fixing the head in a constant vertical position. Participants were asked to keep their eyes well open.

#### TwinsUK

The TwinsUK study proposal was reviewed by the St Thomas’ Hospital, London, England, local research ethics committee, and participants were included after fully informed written consent. The TwinsUK is a volunteer cohort of 10,000 same-sex monozygotic and dizygotic twins recruited from the general population. After performing all quality controls, the present study evaluated 2186 twins for whom unstandardized portrait images were available, including 503 monozygotic twin pairs, 549 dizygotic twin pairs, and 82 single twins. Of these, 15 twin pairs (1.4% of the total sample) were of nonwhite race/ethnicity. All twin pairs \((503 + 549 = 1052)\) were included in the heritability analysis because exclusion of the nonwhite twins gave almost the same heritability estimates. For genetic association analysis, the nonwhite twins were excluded; included were only one random sibling for each monozygotic \((n = 467)\) and dizygotic \((n = 528)\) twin pairs and the single twins \((n = 58)\) for whom genotype data were available after quality controls \((n = 1053)\).

### Phenotyping

Because this is the first epidemiological study to date on sagging eyelids, no well-accepted severity grading scale existed in previous literature. Together with an ocuoplastic ophthalmologist (I.B.), we composed the following 4-level Photonomerical Severity Scale using clearly visible anatomical cutoff points: normal (the upper eyelid skin is not touching the eyelashes), mild (the upper eyelid skin is touching the eyelashes), moderate (the upper eyelid skin is hanging over the eyelashes), and severe (the upper eyelid skin is hanging over the eye), as illustrated using drawings (Figure 1). A physician (L.C.J.) reviewed all photographs and graded eyelid sagging, preferably in the right eye. In the RS, photographs were excluded if the participant blinked (115 photographs), the upper eyelid fold was not fully visible (34 photographs), external traction on the eyelid was perceived (70 photographs), or predefined eyelid or eye conditions were present (24 with ptosis and 39 with exophthalmia), leaving 5578 photographs available for the analysis. In the TwinsUK, photographs were excluded because of ptosis (5 photographs), blinking (29 photographs), a blurry image (5 photographs), a bad head position (1 photograph), or eye trauma (1 photograph), leaving 2186 photographs available for analyses. To validate the newly composed Photonomerical Severity Scale, we followed guidelines for reporting agreement studies. Graders were blinded to the initial grades and regraded a random subset of photographs. The oculoplastic ophthalmologist (L.C.J.) graded at the same time as the initial grades were given, but the initial grader (L.C.J.) regraded a subset 6 months after the initial grading. The intraclass correlation coefficient (consistency-type 2-way mixed model for single measures) was used to assess agreement because this method is commonly recommended for comparison of numerical data. The intrarater agreement between L.C.J. and I.B. and the intrarater agreement of L.C.J. were high.
The interrater intraclass correlation coefficients were 0.88 (95% CI, 0.86–0.90) in the RS (n = 500) and 0.77 (95% CI, 0.68–0.84) in the TwinsUK (n = 100). The intrarater intraclass correlation coefficients were 0.89 (95% CI, 0.82–0.94) in the RS (n = 50) and 0.90 (95% CI, 0.84–0.95) in the TwinsUK (n = 50).

In the RS, data on extrinsic risk factors were collected during home interviews. Questions were asked about the following: (1) current smoking (whether the individual currently smokes pipes, cigars, or cigarettes), (2) poor tanning ability (whether the individual burns easily in the sun), (3) sun protection behavior (whether the individual usually wears sunglasses or a hat with a large brim on sunny days), and (4) hormonal status (whether the woman had a menstrual period in the past year). Skin color was graded visually by a dermatology-trained physician (L.C.J.) as very white, white, or white to olive as described previously.17

Genotyping and Quality Control
In the RS, genotyping was performed using an assay (Infinium II HumanHap550K Genotyping BeadChip, version 3; Illumina). Methods for the collection and purification of DNA have been described in detail previously.18 In brief, single-nucleotide polymorphisms (SNPs) were imputed based on the HapMap Central European-like Utahns (CEU) reference samples (International HapMap Project; http://hapmap.ncbi.nlm.nih.gov) using available software (MaCH; http://www.sph.umich.edu/csg/abecasis/MaCH). In GWAS analysis, SNPs were filtered out if they had a minor allele frequency of less than 1%, a SNP call rate of less than 98%, an individual call rate of less than 95%, and an imputation $r^2$ of less than 0.3. In addition, genotypes were merged with 120 HapMap phase 2 samples, and individuals were removed if they were outside 4 SDs of the principal components of the CEU samples in the multidimensional scaling analysis.13 In total, 2,149,245 SNPs passed quality control. In the TwinsUK, DNA samples were genotyped using a chip (Hap317K; Illumina) and imputed using HapMap CEU samples, and the same filters as for the RS were applied. Quality control at the individual and SNP level has been described in detail previously.19 Herein, we also excluded 6 outlier individuals (>4 SDs) from the TwinsUK based on a multidimensional scaling analysis. In total, 2,263,540 SNPs passed quality control. We also derived the genomic kinship matrices in the RS and the TwinsUK to double-check samples with close relatedness. No individuals were found to have an identical by descent coefficient exceeding 0.15. These quality controls left 5,578 RS and 10,535 TwinsUK unrelated individuals for the GWAS.

Statistical Analysis
Sagging eyelids were graded on a 4-point Photonic Numerical Severity Scale (normal, mild, moderate, or severe). An age- and sex-standardized prevalence of dermatochalasis (moderate and
severe sagging) in the Netherlands was estimated using demographic data from the Dutch population in 2011 (Statistics Netherlands; http://www.cbs.nl/en-GB/menu/home/default.htm).

Risk factors known to be involved in skin aging were analyzed in a sex-stratified multivariable multinomial logistic regression and included age, sex, skin color, tanning ability, current smoking, sun protection behavior, body mass index (BMI), and hormonal status in women. The final model included both sexes because hormonal status in women was not significant. The regression analyses were performed using statistical software (SPSS 20 and SPSS Statistics for Windows, version 20.0; IBM Corporation). These analyses were not performed in the TwinsUK because of missing variables.

In the TwinsUK, differences in the 4 grades of sagging eyelid severity within twin pairs were compared between monozygotic twins (503 pairs) and dizygotic twins (549 pairs) using intraclass correlations. Nonoverlapping CIs indicate a significant difference and suggest genetic influence on sagging eyelids. Heritability analysis was performed with available software (Mx; http://www.vipbg.vcu.edu/tc2012/mxmang30.pdf) using full-information maximum-likelihood estimation of additive genetic variance (ie, heritability), common environmental variance, and unique environmental variance, with sex and age as covariates.

A GWAS was performed in 5578 RS participants and in 1053 TwinsUK participants separately using linear regression adjusted for sex, age, and 4 principal components from the multidimensional scaling analyses. Association was tested for the additive (0, 1, or 2 minor alleles), dominant (0 for wild type or 1 for otherwise), and recessive (1 for homozygote minor allele or 0 for otherwise) models. The inflation factor λ was estimated close to 1.0 in both the RS and the TwinsUK and was not further considered. The distribution of the resultant P values was compared with that under the null hypothesis of no association using a quantile-quantile plot. We used the traditional threshold for genome-wide significance ($P < 5 \times 10^{-8}$), and $P$ values between $5 \times 10^{-8}$ and $5 \times 10^{-6}$ were considered suggestive evidence of association. An inverse-variance meta-analysis was performed on the RS and TwinsUK GWAS results for each genetic model. In addition, we tested the top SNPs ($P < 5 \times 10^{-6}$) from the 3 genetic models in a separate multinomial analysis in the RS and TwinsUK combined (n = 6631) adjusted for age, sex, and cohort status. The effects of the SNPs were estimated using odds ratios (ORs) for the different sagging eyelid severity categories (mild, moderate, and severe) compared with the reference normal control group. The association analyses were conducted using available software (PLINK, version 1.07; http://pngu.mgh.harvard.edu/purcell/plink) and R (http://www.R-project.org).

### Results

Among 5578 RS participants (mean age, 67.1 years; 44.0% male), sagging eyelids were graded as normal in 44.9%, mild in 37.3%, moderate in 12.9%, and severe in 4.9% (Table 1). Moderate and severe sagging eyelids, defined as dermatochalasis, was present in 17.8% of the participants. The overall prevalence of sagging eyelids among individuals 45 years and older in the Netherlands was estimated to be 16.3% (95% CI, 13.1%-19.6%), representing 19.0% (95% CI, 14.1%-23.9%) of men and 14.4% (95% CI, 10.2%-18.6%) of women.

We tested the association between the risk factors and sagging eyelid severity in 5578 RS participants using multivariable multinomial logistic regression (Table 2). Age (per 10 years) was significantly associated with sagging severity. Male sex showed a significant protective effect in the mild group but showed a reversed risk effect in the moderate and severe groups. Lighter skin color demonstrated a significant and consistent risk effect across all 3 severity categories. Higher BMI was significantly associated with mild and moderate sagging eyelids but not with severe sagging eyelids. Finally, current smoking was borderline significantly associated with moderate and severe sagging eyelids. Tanning ability, sun protection behavior, and hormonal status (women only) were not sig-

### Table 1. Characteristics of 5578 Individuals in the Rotterdam Study*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 2455)</th>
<th>Women (n = 3123)</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>66.7 (10.0)</td>
<td>67.3 (10.5)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Severity of sagging eyelids, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>1140 (46.4)</td>
<td>1365 (43.7)</td>
<td></td>
</tr>
<tr>
<td>Mild sagging</td>
<td>809 (33.0)</td>
<td>1271 (40.7)</td>
<td></td>
</tr>
<tr>
<td>Moderate sagging</td>
<td>351 (14.3)</td>
<td>369 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Severe sagging</td>
<td>155 (6.3)</td>
<td>118 (3.8)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Skin color, No. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very white</td>
<td>183 (7.4)</td>
<td>643 (20.6)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1889 (76.9)</td>
<td>2225 (71.2)</td>
<td></td>
</tr>
<tr>
<td>White to olive</td>
<td>383 (15.6)</td>
<td>255 (8.2)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>27.2 (3.6)</td>
<td>27.3 (4.5)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Current smoking, No. (%)</td>
<td>592 (24.1)</td>
<td>599 (19.2)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Easily sunburned, No./total No. (%)</td>
<td>472/1571 (30.0)</td>
<td>667/1984 (33.6)</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>Sun protection behavior, No./total No. (%)</td>
<td>929/1572 (59.1)</td>
<td>1218/2001 (60.9)</td>
<td>.0004</td>
</tr>
<tr>
<td>Menopause, No./total No. (%)</td>
<td>...</td>
<td>2065/2338 (88.3)</td>
<td>...</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); ellipsis, not applicable; No., number of individuals in phenotype subgroups.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10 years)</td>
<td>1.1 (1.0-1.2)</td>
</tr>
<tr>
<td>Sex</td>
<td>0.5 (0.4-0.6)</td>
</tr>
<tr>
<td>Skin color</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>0.6 (0.4-0.9)</td>
</tr>
<tr>
<td>White to olive</td>
<td>1.0 (0.7-1.4)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>1.0 (0.9-1.1)</td>
</tr>
<tr>
<td>Current smoking, No. (%)</td>
<td>0.6 (0.4-0.9)</td>
</tr>
<tr>
<td>Easily sunburned, No./total No. (%)</td>
<td>1.0 (0.7-1.3)</td>
</tr>
<tr>
<td>Sun protection behavior, No./total No. (%)</td>
<td>1.0 (0.7-1.3)</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; CI, confidence interval.
Table 2. Risk Factors Associated With Sagging Eyelid Severity Among 5578 Individuals in the Rotterdam Study*

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Mild Sagging</th>
<th>Moderate Sagging</th>
<th>Severe Sagging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per 10 y</td>
<td>1.05 (1.00-1.11)</td>
<td>1.18 (1.10-1.27)</td>
<td>1.38 (1.25-1.52)</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.79 (0.71-0.89)</td>
<td>1.22 (1.03-1.44)</td>
<td>1.70 (1.31-2.21)</td>
</tr>
<tr>
<td>Lighter skin color</td>
<td>1.16 (1.31-1.03)</td>
<td>1.33 (1.59-1.12)</td>
<td>1.34 (1.74-1.03)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>0.91 (0.79-1.06)</td>
<td>1.20 (0.98-1.47)</td>
<td>1.33 (0.98-1.79)</td>
</tr>
<tr>
<td>Higher BMI</td>
<td>1.03 (1.01-1.04)</td>
<td>1.02 (1.00-1.04)</td>
<td>1.02 (0.98-1.05)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); OR, odds ratio.

* Nonsignificant risk factors were excluded, including tanning ability, sun protection behavior, and hormonal status (women only). Data are from a multivariable multinomial logistic regression analysis comparing normal controls.

Table 3. Association Between rs11876749 and Sagging Eyelids in the Rotterdam Study and the TwinsUK*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>In 5578 Individuals From the Rotterdam Study</th>
<th>Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Control</td>
<td>Mild Sagging</td>
</tr>
<tr>
<td>GG</td>
<td>26.3</td>
<td>27.0</td>
</tr>
<tr>
<td>GC</td>
<td>48.7</td>
<td>51.3</td>
</tr>
<tr>
<td>CC</td>
<td>25.0</td>
<td>21.7</td>
</tr>
<tr>
<td></td>
<td>In 1053 Individuals From the TwinsUK</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>32.2</td>
<td>28.2</td>
</tr>
<tr>
<td>GC</td>
<td>46.6</td>
<td>49.4</td>
</tr>
<tr>
<td>CC</td>
<td>21.2</td>
<td>22.5</td>
</tr>
</tbody>
</table>

* P = 4.3 × 10−9 for the Rotterdam Study, and P = .50 for the United Kingdom Adult Twin Registry (TwinsUK). The P values are from a linear regression genome-wide association study assuming the recessive model. Shown is the frequency of genotypes in the top single-nucleotide polymorphism rs11876749 per sagging eyelid category. G is the major allele, and C is the minor allele. The dominant effect of C is CC plus GC vs GG. The recessive effect of C is CC vs GC plus GG.

Significantly associated with the sagging eyelid severity categories and were not considered further. No statistical interaction between sex and the other risk factors was found. Although all phenotypic characteristics differed statistically significantly between men and women (Table 1), we observed no synergistic effect between these risk factors and sex on sagging eyelid severity, with independent effects seen in our regression model.

Among 1052 TwinsUK twin pairs (mean age, 53.1 years; 53.1% male), sagging eyelids were graded as normal in 52.6%, mild in 31.8%, moderate in 11.8%, and severe in 3.7%. Phenotypic correlation among monozygotic twin pairs (r = 0.65; 95% CI, 0.60-0.70) was on average much higher than that among dizygotic twin pairs (r = 0.37; 95% CI, 0.29-0.44). The variance in sagging eyelids due to additive genetic effect (ie, heritability percentage) was estimated to be 60.9% (95% CI, 43.7%-76.3%) (15.6% showed dermatochalasis). Only 2.2% of the variance was explained by common environmental factors (eg, living in the same family) and 36.9% by other environmental factors. These results demonstrated that the genetic influence of sagging eyelids is nontrivial and suggested that our phenotypic data might be useful for the subsequent genetic association analysis.

In the meta-analysis of our GWAS results, 3 SNPs showed a genome-wide significant association with sagging eyelids for a recessive effect (eFigure 1 in the Supplement) and deviated significantly from the expected null distribution (eFigure 2 in the Supplement). Meta-analysis results demonstrated that all 3 SNPs were located in the same intergenic region on chromosome 18p11, namely, rs4076011 (P = 2.7 × 10−8), rs8096287 (P = 2.1 × 10−8), and rs11876749 (P = 1.7 × 10−8) (eTable 1 in the Supplement). The significance was mostly driven by RS samples (P = 8.7 × 10−8 for rs4076011, P = 4.7 × 10−9 for rs8096287, and P = 4.3 × 10−9 for rs11876749), whereas none were replicated in the TwinsUK. The recessive effect of the top SNP rs11876749 in the RS was evident from the decreasing presence of the CC genotype compared with GG and GC combined in increasing sagging eyelid severity levels (Table 3).

Two genes were located near the intergenic SNP rs11876749; these were DLGAP1 (100 kilobase [kb]) and TGFIF1 (500 kb) (Figure 2). DLGAP1 (Online Mendelian Inheritance in Man [OMIM] 605445) was not reported in previous literature on skin aging, but TGFIF1 (OMIM 602630) encodes an inducer of transforming growth factor β (TGF-β), which is a key factor in skin aging. The top SNP rs11876749 was not in high linkage disequilibrium with SNPs in DLGAP1 and TGFIF1. The possibility that they have a distal regulation effect cannot be excluded given abundant previous examples, such as via long-range chromatin loop formation.

In addition to the 18p11 finding, the meta-analysis under the additive allele effect model identified 28 SNPs with suggestive evidence of association (P < 5 × 10−8). These SNPs were located in 5 different genetic regions containing the following 3 known genes: SMYD2, ATP8A1, and PJA2 (eTable 1 in the Supplement). SMYD3 (P = 4.1 × 10−6 for rs10924350) is involved with metalloproteinase 9 upregulation and MMP9 is a known gene associated with skin aging.

In a multinomial logistic regression model in the RS and TwinsUK combined (n = 6631) (eTable 2 in the Supplement), most SNPs were more significantly associated with moderate and severe sagging eyelids compared with mild sagging eye-
lids. All significantly associated SNPs exerted a consistent protective or risk effect across all 3 sagging eyelid severity categories. The top SNP rs11876749 showed the strongest protective effect for moderate sagging eyelids (OR, 0.54; \( P = 6.1 \times 10^{-9} \)) in the recessive model and was less strong for mild (OR, 0.86; \( P = .02 \)) and severe (OR, 0.67; \( P = .01 \)) sagging.

Discussion
This is the first large, population-based cohort study to date that investigates multiple nongenetic and genetic risk factors for sagging eyelids measured at 4 severity levels. We found that age, male sex, lighter skin color, high BMI, and possibly current smoking are nongenetic risk factors for sagging eyelids. Age has long been recognized as the major risk factor for sagging eyelids.\(^1\) Our data confirmed this knowledge. We also found that men tend to have decreased risk of mild sagging but increased risk of severe sagging, which seems to contradict previous ideas of no sex differences.\(^2\) The observed sex difference is likely explained by multiple biological variations between the sexes, such as hormonal and facial shape differences.\(^3\)\(^9\)\(^10\) Higher BMI has been related to cheek skin wrinkling,\(^6\)\(^20\)\(^21\) indicating that the risk profiles of both conditions are at least partially overlapping.

The GWAS of these 2 independent European population samples revealed one genome-wide significant locus that protects against sagging eyelid severity. This is a recessive effect of the C allele of rs11876749 on chromosome 18. The effect was demonstrated in the RS and showed genome-wide significance in the meta-analysis but was not replicated in the TwinsUK. The lack of replication in the TwinsUK could result from the smaller sample size. Notably, rs11876749 is located less than 500 kB from \( TGIF1 \), which is an inducer of TGF-β.\(^24\) The TGF-β pathway is known to regulate cell cycle progression in fibroblasts, and impaired TGF-β signaling induces skin aging.\(^25\) The SNP rs11876749 is also located close to \( DLGAP1 \); however, this gene is not known to be involved in skin aging.

Among 37 loci showing suggestive evidence of association (\( P < 5 \times 10^{-8} \)) in the RS only, twins only, and meta-analysis of additive, dominant, and recessive allele models, one locus harbors a gene that could be biologically relevant. This is \( SMYD3 \) (OMIM 608783), which is associated with MMP-9 upregulation,\(^27\) and the \( MMP9 \) gene is known to be involved in skin aging\(^28\) as a modulator of the extracellular matrix. Although skin color was found to be a significant risk factor for

The −log\(_{10}\) \( P \) values of all single-nucleotide polymorphisms (small squares) surrounding the most significant signal (large diamond) were plotted against their physical positions on chromosome 18 according to the reference human genome, version 18 (hg18; http://genome-euro.ucsc.edu). Blue peaks represent known recombination rates in HapMap Central European-like Utahns samples (International HapMap Project; http://hapmap.ncbi.nlm.nih.gov) expressed in cM/Mb. The level of redness represents the strength of linkage disequilibrium (\( r^2 \)) of all single-nucleotide polymorphisms in relation to the top single-nucleotide polymorphism. Known genes are aligned below. cM/Mb indicates centimorgan (genetic distance) per megabase (chromosomal distance); kb indicates kilobase.
sagging eyelids, we found no meta-analysis association with well-known skin color genes in the additive allele model, including MCTR (P = .36 for rs1805007), IRF4 (P = .06 for rs1220392), and HERC2 (P = .44 for rs12193832).

Although we detected only one significant locus, which could not be replicated in the second independent cohort, little doubt exists that genetic factors have an important role in sagging eyelids, as evidenced by the high heritability estimated in the TwinsUK sample (up to 60%, similar to the heritability of skin wrinkling). This also suggests that the inheritance of dermatochalasis resembles that of other common complex traits in humans (eg, body height) in that many common DNA variants each have a small effect together in determining the phenotype. However, based on our study design, we cannot exclude the existence of rare variants with larger effects because we focused only on SNPs with a minor allele frequency exceeding 1%.

The main strength of this study is the large population-based sample, with detailed information on possible determinants and outcomes. This information is suitable for studying nongenetic risk factors. In addition, genetic risk factors were studied in 2 independent cohorts, which increases the power and the reliability of our genetic findings. The ascertainment of sagging eyelids in the RS was based on eye photographs, which were initially taken to study the fundus of the eye. Therefore, individuals had been asked to keep their eyes well open, which may have affected the validity and reliability of our case definition. An individual with severe sagging eyelids is more likely to forcefully open the eyes and may have been graded as having moderate or mild severity. This presumed differential misclassification, together with the exclusion of eye photographs that showed signs of external eyelid traction and the fact that we could not exclude individuals who had undergone eyelid surgery because of absence of that information, most likely resulted in an underestimation of the prevalence and the effect sizes of the risk factors. This may also have led to conservative estimations but not to overestimated (false) effects. Despite the available detailed information, our study investigated no other potential dermatochalasis risk factors, including facial shape (eg, deep-set eyes), facial expression differences, or repeated external friction on the eyelid skin. Finally, our study seemed to be underpowered to detect DNA variants with small effects, and the SNPs that we have reported need further replication in other cohorts. Until then, they can be considered only as candidate SNPs. So, enlarging the sample size via global collaboration and including less common DNA variants (eg, via applying next-generation exome or whole-genome sequencing) are indicated for future genetic research on dermatochalasis and general skin aging.

### Conclusions

This study shows that nongenetic risk factors, including age, male sex, lighter skin color, higher BMI, and possibly current smoking, increase the risk of sagging eyelids and demonstrates that the risk profile resembles that of skin wrinkling. The high heritability of sagging eyelids indicates that genetic variants are important in the origin. The C alle of rs11876749 on chromosome 18 showed a genome-wide significantprotective effect for sagging eyelid severity. Future genetic studies are needed to elucidate the mechanisms that explain the interplay between intrinsic and extrinsic factors in the development of skin sagging.

### ARTICLE INFORMATION

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