MMP-1 and -3 Promoter Variants Are Indicative of a Common Susceptibility for Skin and Lung Aging: Results from a Cohort of Elderly Women (SALIA)

Andrea Vierkötter[1], Tamara Schikowski[1,2,3], Dorothea Sugiri[1], Mary S. Matsui[4], Ursula Krämer[1,5] and Jean Krutmann[1,5]

Studies have indicated that there may be a smoking-dependent association between skin wrinkling and airflow obstruction of the lung. It was suggested that this association might be because of an underlying susceptibility in genes responsible for extracellular matrix (ECM) remodeling. Our purpose was to confirm the association between skin wrinkling and airflow obstruction and to identify genetic polymorphisms indicative of an underlying susceptibility. In 697 elderly women, we assessed skin wrinkles by SCINEXA (SCore for INtrinsic and EXtrinsic skin Aging) and airflow obstruction by spirometry, using the ratio of forced expiratory volume in 1 second (FEV_1) to forced volume capacity (FVC). For association analysis, we used multiple regression and found that the FEV_1/FVC ratio decreased 1.2% per 6-point increase in the wrinkle severity score after accounting for age, education, body mass index, skin type, and sun exposure. This association was significant and independent of smoking or air pollution. Most interestingly, this association occurred only in carriers of the matrix metalloproteinase-1 (MMP-1) 2G (rs1799750) or the MMP-3 6A (rs3025058) allele but not in homozygous carriers of the 1G or 5A allele. Thus, skin and lung aging are linked in carriers of the 2G or 6A allele. These alleles appear to be indicative of a common genetic susceptibility.

Journal of Investigative Dermatology (2015) **135,** 1268–1274; doi:10.1038/jid.2015.7; published online 19 February 2015

INTRODUCTION

Aging is a process affecting the entire human body. It is driven by endogenous (Finkel and Holbrook, 2000; Balaban *et al.*, 2005; Blasco, 2005) as well as environmental factors (Steves *et al.*, 2012). As the outermost barrier between the environment and the human body, our skin might be viewed as a model organ for aging. Signs of skin aging might serve as a mirror reflecting internal aging processes of the human body (Makrantonaki and Zouboulis, 2007) with the peculiarity that skin aging signs are directly visible and can be studied noninvasively. This assumption might be particularly true for organs that age as a consequence of extrinsic factors and cellular mechanisms that they hold in common with skin aging. In support of this concept, two previous studies (Lange and Schnohr, 1994 and Patel *et al.*, 2006) showed that there is a smoking-dependent association between skin wrinkling and airflow obstruction of the lung, characteristic aging features of these two organs, respectively. They further suggested that pronounced skin wrinkling might be a marker for an underlying susceptibility for the effect of smoking on airflow obstruction. In addition, Patel *et al.* (2006) hypothesized that changes in the extracellular matrix (ECM) might be important in the pathogenesis of both skin wrinkling and airflow obstruction and that polymorphisms in genes relevant for ECM remodeling might be responsible for the underlying susceptibility of skin and lung aging.

The balance between ECM synthesis and degradation is impaired during both skin (Hornebeck, 2003; Quan *et al.*, 2009) and lung aging (Mocchegiani *et al.*, 2011). In the first case, it results in the formation of wrinkles, whereas in the latter case obstructive pulmonary diseases develop. ECM degradation is attributed to the combined action of several members of the matrix metalloproteinase (MMP) family (Chang and Werb, 2001). Such proteinases are regulated at several molecular levels including transcription, mRNA stability, translational efficiency, enzyme compartmentalization, pro-enzyme activation, or endocytosis. Their activity

[1]*IUF–Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany;* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland;* [3]*University of Basel, Basel, Switzerland and* [4]*Estée Lauder, Melville, New York, USA*

[5]*These authors contributed equally to this work.*

Correspondence: Jean Krutmann, IUF–Leibniz Research Institute for Environmental Medicine, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany. E-mail: Jean.Krutmann@IUF-duesseldorf.de

This study was carried out in Düsseldorf, Germany.

Abbreviations: ECM, extracellular matrix; ETS, (E-twenty-six) transcription factors; FEV_1, forced expiratory volume in 1 second; FVC, forced volume capacity; MMP, matrix metalloproteinase; SALIA, Study on the influence of Air pollution on Lung function, Inflammation and Aging; SCINEXA, SCore for INtrinsic and EXtrinsic skin Aging; TIMP, tissue-specific inhibitor of matrix metalloproteinase

Received 4 June 2014; revised 19 November 2014; accepted 13 December 2014; accepted article preview online 19 January 2015; published online 19 February 2015

in the extra- or pericellular space is mainly regulated by tissue-specific inhibitors designated as TIMPs (tissue-specific inhibitors of matrix metalloproteinase). During skin aging, upregulation of MMP-1 and MMP-3 is responsible for the lysis of dermal collagen and TIMP-1 is decreased with fibroblast senescence (Hornebeck, 2003). Furthermore, many studies have shown that protein and mRNA levels of MMP-1 and MMP-9 are higher in lung tissue and induced sputum of chronic obstructive pulmonary disease patients than of controls (van Diemen *et al.*, 2011).

The purpose of our study was to confirm the association between skin wrinkling and airflow obstruction and its dependence on smoking or other environmental exposures. Furthermore, we asked whether genetic polymorphisms in genes relevant for ECM remodeling are responsible for the underlying susceptibility for skin wrinkling and airflow obstruction.

In order to address these study objectives, we used data from a population-based cohort of elderly women, the SALIA (Study on the influence of Air pollution on Lung function, Inflammation and Aging) study cohort. As likely candidates influencing the susceptibility of skin and lung aging, we investigated several genetic promoter variants in MMP genes: MMP-1 rs1799750 (−1607 1G/2G), MMP-3 rs3025058 (−1612 5A/6A), and MMP-9 rs3918242 (−1562 C/T). These variants have been linked to differential binding of transcription factors (Ye *et al.*, 1996; Rutter *et al.*, 1998; Zhang *et al.*, 1999) and consequently might influence transcriptional activity of the respective gene. In addition, we investigated one genetic variant in the TIMP-1 gene (rs4898 (434 T>C Phe124Phe) that previously had been linked to asthma and excess decline in lung function (van Diemen *et al.*, 2011).

RESULTS

Description of the SALIA study cohort population

Table 1 provides the description of the study population data used in this analysis. Airflow obstruction is a prominent marker for lung aging and is indicated by the ratio of forced expiratory volume in 1 second (FEV_1) to forced volume capacity (FVC). This quotient is well known to linearly decrease with age (Hankinson *et al.*, 2010), and an age and sex standardized ratio below 100% can be used to describe accelerated lung aging. In the SALIA study population, the mean standardized ratio was ∼100% but shows a huge variation between 50 and 134%. Furthermore, skin wrinkling, which is a clinical hallmark of skin aging (Yaar, 2006), and was assessed by a wrinkle severity score of different facial wrinkle types, is presented. The mean wrinkle severity reached a score of 20 in the SALIA study population. Moreover, covariates, which might influence the association between skin and lung aging, are presented, and finally the genotype distributions of the different genetic markers in ECM genes measured in the SALIA study population are described in Table 1.

Association between skin wrinkling and airflow obstruction

We tested the association between skin wrinkling and airflow obstruction adjusted for study center, age, educational level, body mass index, skin type, sunburns in adolescence, and sunbed use (Model 1 in Table 2). A 6-point increase in the wrinkle severity score was associated with a 1.2% (95% confidence interval: −2.3 to −0.1, $P=0.033$) decrease in the FEV_1/FVC ratio, meaning that lung aging was significantly accelerated in those with a higher wrinkle score. In order to test whether the association between skin wrinkling and airflow obstruction depends on smoking or air pollution exposure, we additionally adjusted for smoking or soot exposure in the association analysis. The additional adjustment for the respective environmental factor influenced the change in the FEV_1/FVC ratio per 6-point increase in the wrinkle severity score by <10% (Model 2 and 3 in Table 2). Therefore, the association between skin wrinkling and airflow obstruction is not due to exposure to these environmental factors.

Next, we assessed whether genetic polymorphisms in genes relevant for ECM remodeling are responsible for the underlying susceptibility for skin wrinkling and airflow obstruction. Specifically, we did a stratified analysis according to certain genotype carriers of the measured genetic polymorphisms and calculated the respective *P*-value for interaction (P_{int}) in order to see whether the association is significantly different between the stratified groups (Table 3). We found that certain genotype carriers of the MMP-1 and MMP-3 promoter variant are susceptible to the common development of skin wrinkling and airflow obstruction. In this regard, the association was only pronounced in women having the MMP-1 1G/2G or 2G/2G or the MMP-3 5A/6A or 6A/6A genotype, and there was no association in women having the 1G/1G or 5A/5A genotype, respectively. The association between skin wrinkling and airflow obstruction was not significantly different between certain genotype carriers of the MMP-9 promoter variant or the TIMP-1 variant.

DISCUSSION

This study shows (1) that skin wrinkling was associated with increased lung aging assessed by airflow obstruction, (2) that this association was not due to smoking or soot exposure, but (3) that this association was only pronounced in carriers of the MMP-1 2G or the MMP-3 6A allele and not visible in homozygous MMP-1 1G or MMP-3 5A carriers. These findings indicate that women carrying the MMP-1 2G or the MMP-3 6A allele are particularly prone to develop skin wrinkling as well as accelerated lung aging.

Our findings corroborate and extend previous studies on the association between skin and lung aging (Lange and Schnohr, 1994; Patel *et al.*, 2006). In contrast to previous findings, we could not show that the association between skin and lung aging is smoking dependent. Importantly, we identified genetic polymorphisms that might be responsible for the common underlying susceptibility of skin and lung aging.

Some studies have already suggested that there might be a common genetic susceptibility in genes encoding for proteins involved in pathological mechanisms relevant for both skin and lung aging (Patel *et al.*, 2006). Such common pathological mechanisms might include, for example, changes in collagen and elastin fibers (ECM remodeling) in the skin and the lung.

Table 1. Description of the distribution of airflow obstruction, skin wrinkling, and covariates in the SALIA study cohort population

	N		
Marker of lung aging (airflow obstruction)			
Standardized FEV_1/FVC (%)	697	Mean (min–max)	101.2 (49.7–133.9)
Marker of skin aging			
Wrinkle severity score	697	Mean (min–max)	20.1 (10.0–29.5)
Covariates			
Age (years)	697	Mean (min–max)	73.5 (66.7–79.5)
Highest educational level of the women or her husband	691		
Low (<10 years of school education)		% Yes (*n*)	17.7 (122)
Medium (=10 years of school education)		% Yes (*n*)	48.2 (333)
High (>10 years of school education)		% Yes (*n*)	34.2 (236)
BMI ($kg\,m^{-2}$)	697	Mean (min–max)	27.3 (17.1–45.7)
Sensitive skin type (skin type I or II vs. skin type III or IV according to Fitzpatrick)	697	% Yes (*n*)	55.6 (388)
Sunburns in adolescence	695	% Yes (*n*)	42.6 (296)
Ever sunbed use	697	% Yes (*n*)	18.2 (127)
Smoking	697		
Never smokers		% Yes (*n*)	79.9 (557)
Ever-smokers		% Yes (*n*)	20.1 (140)
High soot exposure ($\geq 2.04\ 10^{-5}\ m^{-1}$ from emission inventory in the year 2000)	694	% Yes (*n*)	25.1 (174)
Genetic polymorphisms in ECM genes			
MMP1 rs1799750 genotypes	697		
1G/1G		% Yes (*n*)	31.6 (220)
1G/2G		% Yes (*n*)	49.2 (343)
2G/2G		% Yes (*n*)	19.2 (134)
MMP-3 rs3025058 genotypes	672		
5A/5A		% Yes (*n*)	25 (168)
5A/6A		% Yes (*n*)	50.5 (339)
6A/6A		% Yes (*n*)	24.5 (165)
MMP-9 rs3918242 genotypes	673		
C/C		% Yes (*n*)	73.1 (492)
C/T		% Yes (*n*)	25.3 (170)
T/T		% Yes (*n*)	1.6 (11)
TIMP-1 rs4898 genotypes	689		
T/T		% Yes (*n*)	26.3 (181)
T/C		% Yes (*n*)	49.3 (340)
C/C		% Yes (*n*)	24.4 (168)

Abbreviations: BMI, body mass index; ECM, extracellular matrix; FEV_1/FVC, ratio of forced expiratory volume in 1 second to forced volume capacity; MMP, matrix metalloproteinase; SALIA, Study on the influence of Air pollution on Lung function, Inflammation and Aging; TIMP-1, tissue-specific inhibitor of matrix metalloproteinase-1.

Up to now, however, it was not known which genetic variants might be responsible. The results of this study indicate that the MMP-1 promoter variant (rs1799750, −1607 1G/2G) as well as the MMP-3 promoter variant (rs3025058, −1612 5A/6A) might represent such genetic factors.

MMP-1 (collagenase) degrades the interstitial type I, II, and III collagens. Graves (1998) showed that the MMP-1 2G allele is of functional relevance as it creates a binding site for transcriptional factors of the E-twenty-six (ETS) family. At this ETS-binding site, members of the ETS transcriptional factor family are able to bind and influence the transcriptional activity of the MMP-1 gene. Rutter *et al.* (1998) showed that the 2G allele displays significantly higher transcription in normal fibroblasts and melanoma cells than the 1G allele that, in consequence, might lead to higher MMP-1 mRNA levels. Indeed, this assumption is supported by more recent studies

Table 2. Association between skin wrinkling and airflow obstruction

	% Change of the FEV_1/FVC ratio per 6-point increase in wrinkle severity score	95% CI	*P*-value
Model 1: Adjusted for study center, age, educational level, BMI, skin type, sunburns in adolescence, and sunbed use	−1.2	−2.3 to −0.1	0.033
Model 2: Model 1 additionally adjusted for smoking	−1.2	−2.3 to −0.1	0.035
Model 3: Model 1 additionally adjusted for soot exposure	−1.1	−2.2 to 0.04	0.059

Abbreviations: BMI, body mass index; CI, confidence interval; FEV_1/FVC, ratio of forced expiratory volume in 1 second to forced volume capacity.
Change of the FEV_1/FVC ratio in percent per 6-point increase in the skin wrinkle severity score adjusted for further influencing factors (confounding by smoking or air pollution is tested by additional adjustment for these factors).

Table 3. Testing for genetic susceptibility for skin wrinkling and airflow obstruction

	% Change of the FEV_1/FVC ratio per 6-point increase in wrinkle severity score	95% CI	*P*-value	P_{int}
MMP-1				
1G/1G	0.3	−1.7 to 2.2	0.800	0.048
1G/2G and 2G/2G	−1.9	−3.3 to 0.5	0.008	
MMP-3				
5A/5A	0.4	−1.7 to 2.5	0.697	0.020
5A/6A and 6A/6A	−2.1	−3.4 to −0.8	0.002	
MMP-9				
C/C	−1.6	−3.0 to −0.2	0.022	0.485
C/T and T/T	−0.7	−2.7 to 1.4	0.531	
TIMP-1				
T/T	−2.5	−4.9 to −0.04	0.050	0.425
T/C and C/C	−0.8	−2.1 to 0.5	0.209	

Abbreviations: CI, confidence interval; FEV_1/FVC, ratio of forced expiratory volume in 1 second to forced volume capacity; MMP, matrix metalloproteinase; P_{int}, *P*-value for interaction; TIMP-1, tissue-specific inhibitor of matrix metalloproteinase-1.
Change of the FEV_1/FVC ratio in percent per 6-point increase in the skin wrinkle severity in certain genotype carriers of MMP-1, -3, and -9 and TIMP-1 genetic variants (P_{int} indicates whether the difference between certain genotype carriers is significant or not).

showing that the 2G allele is associated with higher MMP-1 protein levels in comparison to the 1G allele in blood serum (Huang *et al.*, 2013; Sri Manjari *et al.*, 2013). As the frequency of the 2G allele was increased in tumor cell lines, Rutter *et al.* (1998) suggested that cells expressing the 2G allele might provide a mechanistic explanation for more aggressive matrix degradation, thereby facilitating cancer progression. Regarding the association between skin wrinkling and airflow obstruction, this would mean that an inherent more aggressive matrix degradation by MMP-1 confers a common susceptibility for the two aging processes.

MMP-3 (stromelysin-1) is capable of degrading proteoglycan, fibronectin, laminin, and type IV collagene and may activate other MMPs including MMP-1 (Johansson *et al.*, 2000). It has been shown that atherosclerosis patients homozygous for the 6A allele show a more rapid progression of the disease (Ye *et al.*, 1996). Furthermore, in transient transfection experiments, a stromelysin-1 promoter construct with 6A at the polymorphic site was found to express less of the chloramphenicol acetyltransferase reporter gene than the construct containing 5A (Ye *et al.*, 1996), meaning that the 6A allele is associated with a lower transcriptional activity. These results indicate that not a higher MMP-3 transcriptional activity, as suggested for the MMP-1 gene, but a lower transcriptional activity is associated with common chronic connective tissue disorders. This reinforces the paradigm that ECM remodeling is a complex process including ECM synthesis and degradation.

The MMP-9 rs3918242 and TIMP-1 rs4898 genetic polymorphism were not associated with the underlying genetic susceptibility of the common development of skin and lung aging. MMP-9 (gelatinase B) has a broad substrate specificity, being particularly active against gelatins (denaturated collagens that have lost the typical triple helix) and type IV collagen. It also possesses proteolytic activity against proteoglycan core protein and elastin. Transient transfection experiments and DNA–protein interaction assays indicated that the MMP-9T allele had a higher promoter activity than the C allele, and this appeared to be due to preferential binding of a putative transcription repressor protein to the C allele promoter (Zhang *et al.*, 1999). However, in our study, the MMP-9T

allele was not associated with a higher susceptibility for skin and lung aging in comparison to the C allele. The TIMP-1 rs4898 genetic variant is located in the region responsible for binding and inactivating MMP-9 and has been associated with asthma and an excess decline in FEV_1 (van Diemen *et al.*, 2011). However, it is a genetic variant resulting in a synonymous amino acid change (Phe124Phe), and thus its functional importance is not clear up to now.

These findings might further suggest that especially genetic variation in MMP-1 and MMP-3 genes and its related functional consequences are important for the underlying genetic susceptibility and that genetic variation in MMP-9 and TIMP-1 does not further contribute to the genetic susceptibility.

Limitations of the SALIA study include mortality and loss to follow-up during the 20-year follow-up, resulting in a lower rate of participation at follow-up relative to baseline and further to a population of healthy survivors. The observed prevalence of skin wrinkling or airflow obstruction may be affected by this loss to follow-up or healthy survivor bias and might not be representative for the general female population. Furthermore, the power of our study is limited because of the available sample size, and therefore we might have missed significant associations. We have restricted our analysis to four promising genetic polymorphisms in genes involved in ECM remodeling. However, ECM remodeling is a very complex process, including a large set of MMPs and TIMPs as well as further regulating factors, and the combined genetic variation in all these genes is most probably influencing the common susceptibility of skin and lung aging.

On the other hand, the strength of our study is that we have a relatively high number of elderly women with a homogenous environmental, lifestyle, and genetic background.

In conclusion, skin wrinkling and airflow obstruction are linked in carriers of the MMP-1 2G allele as well as in carriers of the MMP-3 6A allele in elderly women of the SALIA study cohort. These alleles appear to be indicative of a common genetic susceptibility for skin and lung aging.

MATERIALS AND METHODS

Study design and study participants

The SALIA study was initiated as a cross-sectional study between 1985 and 1994 as part of the Environmental Health Survey, an element of the Clean Air Plan introduced by the Government of North-Rhine Westphalia in Germany. A detailed description of the SALIA study has been previously provided by Schikowski *et al.* (2005). The study areas were chosen from the Ruhr district in Germany and two rural counties north of the Ruhr district in order to represent a range of exposures to airborne particulate matter from traffic and steel and coal industries. In the baseline investigation, all women aged 54–55 years living in predefined areas were asked to participate. In Figure 1, a flowchart presents the development of the SALIA study cohort from baseline to follow-up. In this study, we refer to the participants of the follow-up investigation from 2007 to 2010. Of all participants at the follow-up investigation, 697 women participated in the skin examination, lung function measurement, and body fluid sampling for the determination of genetic polymorphisms. The Medical Ethics Committee of the Ruhr University Bochum (Bochum Germany) approved the follow-up study. The Declaration of Helsinki Principles was followed, and all study participants were informed in detail by written form and gave written consent.

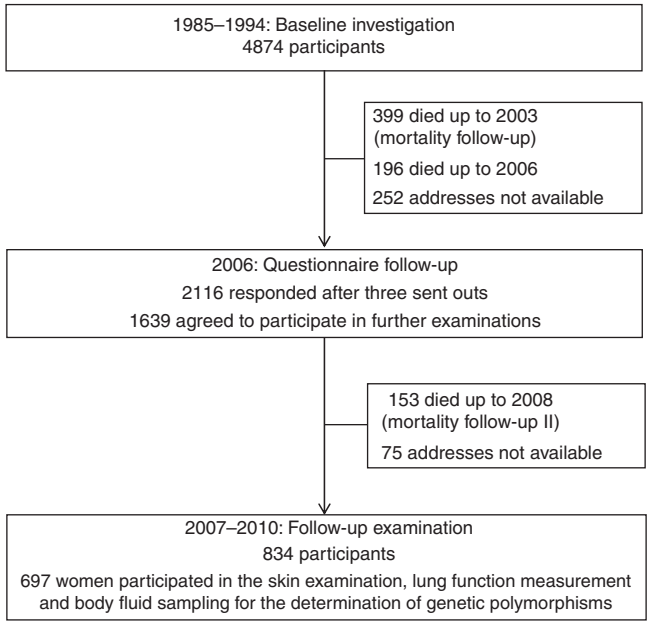

Figure 1. Flowchart of SALIA (Study on the influence of Air pollution on Lung function, Inflammation and Aging) study follow-up.

Measurement of airflow obstruction as a marker of lung aging

We used airflow obstruction as a marker for lung aging. Airflow obstruction was measured spirometically according to the ATS/ERS (American Thoracic Society/European Respiratory Society) recommendations (Miller *et al.*, 2005). This means that eight measurements at maximum were performed under direction of trained personnel, and the values where FEV_1 was reached were used. The spirometer was calibrated before each testing, and all flow–volume curves were reviewed visually by a pulmonary physician. Spirometric tests, which did not hold the ATS/ERS guidelines and were not visually acceptable, were excluded. Finally, airflow obstruction was defined using the FEV_1/FVC ratio that was further age and sex standardized according to the reference values by Hankinson *et al.* (2010) and was expressed in percent. A standardized FEV_1/FVC ratio of 100% means that the lung function equals the lung function of healthy women with the same age and height. A lung function of $>100\%$ is better and a lung function of $<100\%$ is worse than the lung function of healthy women with same age and height. The FEV_1/FVC ratio is well known to linearly decrease with age (Hankinson *et al.*, 2010), and an age and sex standardized ratio below 100% can be used to describe accelerated lung aging.

Evaluation of skin wrinkling

We determined skin aging by applying SCINEXA (SCore for INtrinsic and EXtrinsic skin Aging, Vierkötter *et al.*, 2009, 2010). The SCINEXA is a protected trademark and the copyright is owned by the IUF (represented by JK). The severity of facial skin wrinkling was evaluated separately on the forehead, between the eyebrows (frown lines), in the crow's feet area, under the eyes, on upper lip, and

nasolabial according to photoreference scales from 0 (not present) to 5 (very severely present) (Tschachler and Morizot, 2006). For the overall wrinkle severity score, we summed up the single wrinkle-type severities.

Assessment of covariates

Covariates that might influence skin aging and lung aging like age, educational level, body mass index, smoking, skin type, and sun exposure (sunburns in adolescence and sunbed use) were assessed by questionnaire. Another known covariate is exposure to air pollution. In 2010, we could show that soot exposure in particular is associated with increased skin aging (Vierkötter *et al.*, 2010), and therefore soot exposure was selected as an indicator for air pollution exposure. Soot concentration from traffic-related sources was estimated by the blackness of fine particle filters and was then assigned to each individual's address by land-use regression models (Hochadel *et al.*, 2006). This exposure assessment was identical to that employed in the TRAPCA (Traffic-Related Air Pollution and Childhood Asthma) study (Brauer *et al.*, 2003; Cyrys *et al.*, 2003). We defined high soot exposure, if the subject has a soot value $\geq$75% percentile of the soot distribution. In order to investigate the underlying genetic susceptibility for skin winkling and airflow obstruction, four genetic markers in ECM genes (MMP-1 rs1799750, MMP-3 rs3025058, MMP-9 rs3918242, and TIMP-1 rs4898) were measured in isolated DNA out of body fluid cells (blood or buccal swabs) by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Jaremko *et al.*, 2005). A very low percentage (<5%) of samples could not be measured. The distribution of the genetic markers was in Hardy–Weinberg equilibrium.

Statistical approach

Airflow obstruction was defined as the dependent variable (=outcome), and we thus tested the influence of increasing skin wrinkling on airflow obstruction. As airflow obstruction defined by the FEV_1/FVC ratio is a continuous variable with a normal distribution (Supplementary Figure S1 online), we applied multiple linear regression analysis. The respective formula of our regression analysis is described in the Supplementary Materials and Methods S2 online.

In the first step, we analyzed the influence of increasing skin wrinkling severity on airflow obstruction adjusted for study center, age, educational level, body mass index, skin type, and sun exposure. The result of this linear regression analysis is the percent change in the standardized ratio FEV_1/FVC per 1-unit change in the wrinkle score. The unit of the wrinkle score was defined by its interquartile range, which is the range from the 25th percentile to the 75th percentile and which corresponds to 6-score points in the SALIA study population.

In the next step, we analyzed whether the association between skin wrinkling and airflow obstruction depends on smoking or air pollution exposure (confounding). For this purpose, we included smoking or soot exposure as additional covariate in the linear regression model. A confounding effect is present if the inclusion of one of these covariates reduces the influence of skin aging severity on airflow obstruction of >10%.

In the final step, we assessed whether genetic polymorphisms in genes relevant for ECM remodeling are responsible for the underlying susceptibility for skin wrinkling and airflow obstruction. For this purpose, we tested whether the association is significantly different between stratified groups according to certain genotypes by calculating the *P*-value of interaction (P_{int}). The P_{int} can be calculated by including multiplicative interaction terms in the linear regression model.

An association was defined as significant if the *P*-value in the respective linear regression model was <0.05.

The statistical analysis was performed with SAS 9.2 (SAS/STAT Software; SAS Institute, Cary, NC, 2002–2003). For multiple linear regressions, we used the mixed procedure and the estimates were gained by maximum likelihood.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

The SALIA follow-up study was funded by the German Statutory Accident Insurance (DGUV). The skin aging measurement was supported by an unrestricted grant from the Estée Lauder Companies and by the Deutsche Forschungsgemeinschaft (DFG Kr 871/5-1). Road maps with emissions from traffic and particulate matter values from measurement stations were maintained from the State Environment Agency of North Rhine Westphalia (LANUV). We thank Dr Christine Müller-Brandes for visually reviewing all flow–volume curves. We also thank all study participants.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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