healing does not augment WIHN in these IL-6 KO mice (data not shown) as it does in WT mice (Nelson et al., 2015), suggesting that STAT3 pathway activation is already sufficient for WIHN and WIHN cannot be enhanced further. Dimerization with gp130 to elicit activation of the JAK/STAT3 pathway is common to IL-6 family members. Ciliary neurotrophic factor and leukemia inhibitory factor have also been linked to tissue regeneration, suggesting that the activity of gp130 is the critical key player in this pathway (Heinrich et al., 2003).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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Traffic-Related Air Pollution Contributes to Development of Facial Lentigines: Further Epidemiological Evidence from Caucasians and Asians

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TO THE EDITOR

Skin integrity is compromised by air pollution (Krutmann et al., 2014). Chronic exposure to traffic-related particulate matter (PM) was previously linked to development of facial lentigines in 400 Caucasian women from the Study on the Influence of Air Pollution on Lung Function, Inflammation and Aging (SALIA) cohort study. In addition to PM, traffic-related air pollution is characterized by increased concentrations of nitrogen dioxide (NO_2). NO_2 exposure is known to be associated with low lung function and lung cancer (Adam et al., 2015; Hamra et al., 2015). The effects of NO_2 on human skin have never been investigated. Because environmentally induced lung and skin aging appear to be closely related (Vierkötter et al., 2015), we assessed the link between chronic exposure to NO_2 and lentigo development. Data from an extended SALIA population and an independent study of Han Chinese from the Chinese Taizhou cohort were analyzed.

Both cohort studies are described in detail elsewhere (Schikowski et al., 2010; Vossoughi et al., 2014; Wang

Abbreviations: PM, particulate matter; PM_{10}, particulate matter of <10 μm in diameter; SALIA, Study on the Influence of Air Pollution on Lung Function, Inflammation and Aging

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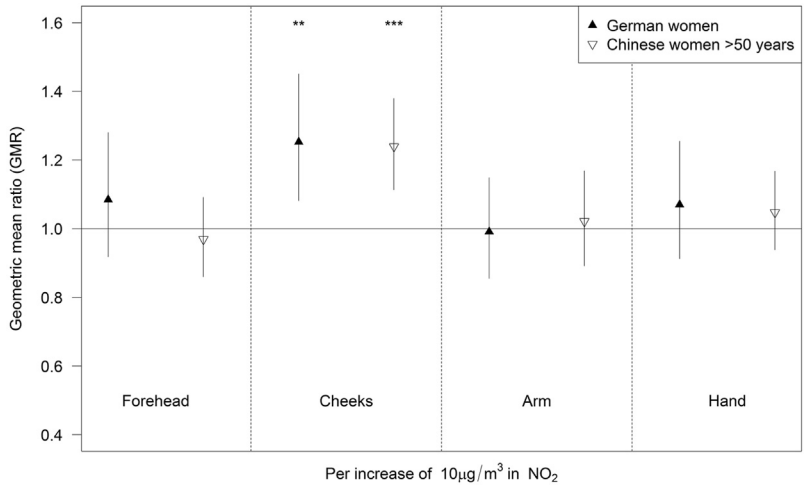

Figure 1. Association between an increase of 10 µg/m³ in NO_2 exposure and the relative amount of lentigines in women from the German SALIA cohort and in women older than 50 years from the Chinese Taizhou cohort. All models were adjusted for age, BMI (kg/m²), smoking history, passive smoking, socioeconomic status and daily average sun exposure during adult life. In the analysis of the SALIA population we adjusted additionally for skin type (skin type I or II vs. skin type III or IV according to Fitzpatrick), sunbed use (yes or no), time of sun bathing and the use of sun protection. In the analysis of the Taizhou cohort we adjusted additionally indoor air pollution exposure (coal or biomass heating). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

et al., 2009). In this study, lentigines were visually evaluated by trained personnel according to photo reference scales (Tschachler and Morizot, 2006) and on the basis of the validated skin aging score SCINEXA (Vierkötter et al., 2009; Vierkötter et al., 2010) in a highly standardized manner as described by Li et al. (2015). Lentigines were valued as follows: 0 indicated no spots, 5 indicated 1−10 spots, 30 indicated 11−50 spots, and 75 indicated more than 50 spots. In SALIA, air pollution exposure was estimated with land-use regression models according to the European Study of Cohorts for Air Pollution Effects (ESCAPE) study (Beelen et al., 2013; Eeftens et al., 2012). In the Taizhou cohort, data from monitoring stations provided by the Taizhou Environmental Bureau were used. Linear regression models adjusted for potential confounders were used to analyze the association between NO_2 exposure and the relative amount of lentigines (Figure 1). Adjusted regression coefficients were transformed to geometric mean ratios with 95% confidence intervals. Statistical computing was performed with R version 3.1.2 (Foundation for Statistical Development, Vienna, Austria).

Table 1. Study characteristics of the German SALIA cohort and the Chinese Taizhou cohort

Characteristic	SALIA	Taizhou
N	806	1072
Female, n (%)	806 (100.0)	743 (69.3)
Age (in y), AM (min−max)	73.5 (66.7−79.8)	59.0 (27.9−89.7)
<10 y of education, n (%)	143 (17.7)	883 (82.4)
BMI, AM (95% CI)	27.3 (27.0−27.6)	24.0 (23.8−24.2)
Ever smoked, n (%)	162 (20.1)	231 (21.5)
Pack years, AM (95% CI)	3.9 (3.0−4.7)	6.0 (5.1−6.8)
Passive smoking, n (%)	486 (60.3)	437 (40.8)
Average daily sun exposure during lifetime (in h), AM (95% CI)	2.6 (2.5−2.7)	3.5 (3.4−3.6)
Average yearly time of sun bathing (in h), AM (95% CI)	8.4 (7.0−9.8)	NA
Skin type I or II, n (%)[1]	455 (56.5)	NA
Sunbed use, n (%)	145 (18.0)	NA
Use of cosmetic with sun protection factor, n (%)	491 (60.9)	45 (4.2)
Coal/biomass heating, n (%)	75 (9.3)	480 (44.8)
NO_2 level (µg/m³;),[2] mean (SD)	28.80 (7.84)	24.06 (6.16)

Abbreviations: AM, arithmetic mean; BMI, body mass index; CI, confidence interval; max, maximum; min, minimum; NA, not applicable; SD, standard deviation.
[1]Skin type from type I to IV according to Fitzpatrick was assessed only in the SALIA study population.
[2]SALIA cohort: air pollution exposure was estimated with land-use regression models according to the procedure developed in the European Study of Cohorts for Air Pollution Effects (ESCAPE) study; Taizhou cohort: air pollution exposure was obtained from state monitoring stations.

Table 1 shows a brief description of the study characteristics and Supplementary Tables S1 and S2 (online) show a description of number of lentigines in both cohorts. Mean levels of NO_2 exposure were 28.8 µg/m³ in the SALIA study area and 24.1 µg/m³ in the Taizhou study area.

Exposure to NO_2 was significantly associated with more lentigines on the cheeks in both cohorts (Figure 1, see Supplementary Table S3 online). In SALIA, an increase of 10 µg/m³ in NO_2 was associated with 25% more lentigines on the cheeks ($P = 0.003$). The strongest association between NO_2 and lentigines was on the cheeks of women older than 50 years (Figure 1, see Supplementary Table S3). This association was identical to the one observed in elderly German women from the SALIA study. Accordingly, in Chinese women older than 50 years, an increase of 10 µg/m³ in NO_2 was associated with 24% more lentigines on the cheeks ($P < 0.001$).

Of note, no association was observed with lentigines on the dorsal hands and forearms, further strengthening the concept that the pathogenesis of lentigines might differ depending on anatomical site. In a first sensitivity analysis we studied the association between NO_2 and PM of <10 µm in diameter (PM_{10}) on lentigines in two separate models (see Supplementary Figure S1 online). This analysis showed that NO_2 had a slightly stronger effect on lentigines than did PM_{10}. In a second sensitivity analysis we analyzed two-pollutant models that included NO_2 and PM_{10} (see Supplementary Figure S2 online). The effects of NO_2 and PM_{10} were reduced

and not significant anymore. This shows that the effects of NO_2 and PM_{10} cannot be disentangled because of high correlation between these measures. These analyses were performed in SALIA only, where individual exposure measures were available.

To the best of our knowledge this is the largest epidemiological study showing a link between traffic-related air pollution and the formation of lentigines. Furthermore, we showed the association not only in Caucasians but also in Asians, a population in which lentigo formation is considered to be a hallmark of skin aging.

We previously reported the association between traffic-related soot exposure and increased lentigo formation (Vierkötter et al., 2010). Soot, which frequently results from diesel exhaust, is a mixture of carbon particles covered by organic compounds including polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbon-induced activation of aryl hydrocarbon receptor signaling in epidermal cells might provide a mechanistic explanation for these epidemiological observations (Krutmann et al., 2014). Indeed, stimulation of primary human epidermal keratinocytes with ambient soot was recently shown to cause an activation of the aryl hydrocarbon receptor and subsequent gene transcription (Nakamura et al., 2015). Whether similar mechanisms are also involved in gaseous pollutant-induced lentigo formation is currently not known. In fact, no studies have been conducted to assess the effects of NO_2 on epidermal cells. There is evidence, however, for proinflammatory effects of NO_2 on human bronchial epithelial cells (Ayyagari et al., 2007).

Several studies found an effect of NO_2 on the lung (Adam et al., 2015). In these studies NO_2 mostly served as a marker for a mixture of pollutants formed in the high temperature combustion of fossil fuels, including fine and ultrafine particles. Further mechanistic studies are needed to clarify whether NO_2 is one of the causal substances inducing the effect.

Strengths of this study are the use of two large ethnically different cohorts (Caucasians and Asians), centrally defined harmonized variables about living conditions and sun exposure, and the use of the SCINEXA to evaluate skin aging in both study populations. There are also some limitations. Air pollution data were obtained from state monitoring stations in the Taizhou cohort; therefore, individual assignment of exposure was fairly coarse leading to small spatial variation. Furthermore, in the SALIA study there might be higher nonsystematic errors in the individual exposure data. Nevertheless, the similar effect estimates in both studies indicate a true underlying effect.

In conclusion, our results corroborate and extend our previous notion that exposure to traffic-related air pollutants influences the formation of lentigines. They indicate that this conclusion is relevant for Caucasians and Asians and that air pollution-associated gases contribute to this effect.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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Intronic *ITGA3* Mutation Impacts Splicing Regulation and Causes Interstitial Lung Disease, Nephrotic Syndrome, and Epidermolysis Bullosa

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TO THE EDITOR

Loss-of-function mutations of the gene for the integrin α3 *(ITGA3)* subunit were recently associated with a rare autosomal recessive multiorgan disorder comprising interstitial lung disease, nephrotic syndrome, and junctional epidermolysis bullosa (ILNEB, OMIM#614748) (Has et al. 2012). Although integrin α3β1 is known to be an essential integrin in epithelia, including epidermal keratinocytes, alveolar epithelial cells, and podocytes, the clinical features of the human disorder and the underlying molecular pathomechanisms remain poorly understood (Nicolaou et al., 2012; Sachs and Sonnenberg, 2013; Shukrun et al., 2014; Yamada and Sekiguchi, 2013).

Here, we report on a patient with ILNEB due to an unconventional *ITGA3* intronic mutation and show that the pathogenic mechanism involves generation of a new acceptor splice site and of a new interaction site with a splicing regulatory element.

The diagnosis of ILNEB was suspected in a 4-month-old male patient, first born to healthy, consanguineous Pakistani parents with no significant family history, because of the concomitant presence of interstitial lung disease with persistent respiratory distress and renal involvement. As the child was critically unwell with no clear diagnosis, it was important to make a precise and rapid diagnosis to inform prognosis and therapeutic decision making. Thus, in a candidate gene approach, *ITGA3* mutation analysis was performed (Has et al., 2012), after written informed consent and ethical approval. This analysis revealed in the patient a single homozygous unclassified variant, c.1383-11T>A, in intron 9 of *ITGA3* (NM_002204.2, NC_000017.11). This variant was present in a heterozygous state in each of the parents (Figure 1a). In addition, it was excluded from 200 control chromosomes and from databases (single nucleotide polymorphism database 141, Exome Variant Server).

Given the lack of skin findings in the child at this stage and intronic nature of the variant, it was necessary to substantiate the disease-causing role of this unclassified variant. To expedite the molecular diagnosis, a skin biopsy was obtained from the patient for additional protein and RNA studies. For immunofluorescence staining, a panel of antibodies to proteins of the dermal-epidermal junction zone was used, including the integrin α3 primary antibody P1B5 (Millipore, Darmstadt, Germany) (Has et al., 2012). Surprisingly, although clinically no cutaneous fragility was noted, the biopsy taken from an unaffected skin area after gentle rubbing displayed multiple junctional splits. These were indicated by the presence of immunoreactivity for integrin α6 at the blister roof and for laminin α3 and collagen VII at the blister base (Figure 1b). In the patient's skin, there was an absence of immunoreactivity for integrin α3, which stained at the periphery of basal keratinocytes in control skin (Figure 1b). Thus the clinical suspicion was confirmed on a molecular level. By this point, the child had an acute respiratory deterioration and did not survive. Examination just prior revealed the presence of blisters, confirming the skin phenotype associated with ILNEB.

To explain the pathogenic role of the identified unclassified variant, we analyzed *ITGA3* transcripts expressed in the skin of the patient and a healthy age-matched control. Total RNA was isolated from skin sections using an RNAeasy FFPE kit (QIAGEN, Hilden, Germany), transcribed into cDNA (Fermentas, St. Leon-Rot, Germany),

Abbreviations: ITGA3, integrin α3 gene; ILNEB, interstitial lung disease, nephrotic syndrome, epidermolysis bullosa

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