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Follow-up on genome-wide main effects: Do polymorphisms modify the air pollution effect on lung function decline in adults?

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ABSTRACT

Improved air quality has been found associated with attenuated age-related decline in lung function. But whether genetic polymorphisms strongly associated with lung function play a modifying role in this attenuation process has so far not been investigated.

We selected ten single nucleotide polymorphisms derived from the largest genome-wide association studies on lung function and examined whether they modified the association between the change in exposure to particulate matter ≤10 μm (ΔPM_{10}) and lung function decline. 4310 participants from the SAPALDIA cohort provided valid spirometry measurements, a detailed pulmonary health questionnaire both at baseline and 11 years later as well as blood samples for genetic testing. Spatially and temporally resolved air pollution exposures were assigned on an individual level based on participants' residences.

Statistically significant interactions of moderate strength with ΔPM_{10} were detected for rs2284746. Individuals with the CC genotype had a 21 ml slower annual decline of the mid expiratory flow per 10 μg/m^3 PM_{10} reduction over an 10-year period, while the benefits of CG and GG carriers were smaller (14 and 7 ml per year, respectively; $P_{interaction} = 0.04$). The attenuated annual decline in the percentage of the forced expiratory volume in one second relative to the forced vital capacity (FEV_1/FVC) was also increased with the presence of each C-allele ($P_{interaction} = 0.009$). We observed further suggestive interactions of similar magnitude in never-smokers, but none of the results would remain statistically significant after correction for multiple testing.

We could not find strong evidence that lung function benefits from improved air quality are modified by polymorphisms associated with lung function level in large meta-analyzed genome-wide association studies.

1. Introduction

Air pollution, a term describing a complex mixture of chemicals and particles in the ambient air, could be linked to several adverse health outcomes and chronic diseases (Chen et al., 2008). Adverse short-term effects of air pollution on respiratory health are well documented (Brunekreef et al., 1995), and evidence is also strong for a long-term effect of air pollution on slowing down growth of lung function in children. This results in deficits by the time respiratory function starts to level off and ultimately decline (Gotschi et al., 2008). Evidence for adverse long-term effects of air pollution on lung function in adults is more limited (Kunzli et al., 2010). In the Swiss Study on Air Pollution and Lung Disease in Adults (SAPALDIA), a reduction of particulate matter smaller than 10 μm in diameter (PM_{10}) resulted in an attenuation of age-related decline of two lung function measures, namely forced expiratory volume in the first second (FEV_1), as well as forced expiratory flow between 25 and 75% of the forced vital capacity (FEF_{25-75}) (Downs et al., 2007).

Since susceptibility to air pollution appears to differ among subjects of the general population, the individual genetic background is likely to play a role in the response to inhaled pollutants. Gene–environment interaction studies have been hence carried out to find such genes which alter the association between air pollution and pulmonary disease or cardiovascular outcomes (Canova et al., 2012; Zanobetti et al., 2011). Studies on lung function have concentrated so far on candidate genes which play a role in the response to free radicals (oxidative stress) (Curjuric et al., 2010; Minelli et al., 2011), in inflammatory processes (Yang et al., 2005), and in cell cycle regulation (Imboden et al., 2009), assigning effect modification to variations in the genes *NQO1*, *HMOX1*, *GSTM1*, *GPX1*, *TNF*, *LTA*, *CCND1*, and *TP53*.

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These single candidate genes had been selected based on biological knowledge, but a more comprehensive investigation of pathways involving several inflammatory and oxidative stress genes led to limited results at either the gene or pathway level, including a suggestive interaction of the *CRISP2* gene with the change in PM_{10} on lung function decline (Curjuric et al., 2012).

A large meta-analysis of 23 genome-wide association studies (GWAS) on lung function yielded 34 single nucleotide polymorphisms (SNPs) pointing to 29 different genetic regions that showed new evidence of association with FEV_1 or with the ratio between FEV_1 and the forced vital capacity (FVC) (Soler Artigas et al., 2011). In the replication phase, the ten regional lead SNPs with the lowest P-values were genotyped and analyzed in nine studies including SAPALDIA. We investigated whether these polymorphisms might represent candidate genes with the potential to modify the effect of air pollution exposure on lung function decline, a phenotype known to be influenced by environmental exposures like air pollution or occupational exposure.

2. Materials and methods

2.1. Study population and sample size

Details about the design of the SAPALDIA cohort study have been described elsewhere (Ackermann-Liebrich et al., 2005; Martin et al., 1997). Briefly, a random population sample of adults from eight areas in Switzerland was examined in 1991 (baseline) with a detailed health questionnaire and spirometry measurements. 8047 out of 9651 were followed-up 11 years later and 6058 agreed to provide blood samples for genetic testing. For the current analysis, we excluded 784 subjects because of incomplete or invalid spirometry at either time point, 30 subjects because they had lived for less than one year at their last residential address at follow-up or could not be assigned PM_{10} values for other reasons, and 934 subjects due to missing covariate data. This resulted in a sample size of 4310 individuals. Selection bias due to the large number of subjects with missing covariate data was addressed by weighting each observation inverse to the probability of being included in the final study sample. Due to unsuccessful genotyping (see below), the sample size was further reduced by a minimum of 22 (rs2857595) and a maximum of 41 (rs1551943) subjects (Table S1). Ethical approval was obtained from the Swiss Academy of Medical Sciences and the Regional Ethics Committees. Written informed consent was obtained from all participants before health examination and biological sample collection at both surveys.

2.2. Spirometry

Spirometry was performed without bronchodilation and according to the European Community Respiratory Health Survey protocol (Burney et al., 1994) and complied with American Thoracic Society criteria (1995). Identical spirometry protocols and devices (Sensormedics model 2200, Yorba Linda, USA) were used in 1991 and 2002 (Kunzli et al., 2005). At least two acceptable and reproducible measurements of FVC and FEV_1 were obtained. In the present study, we studied annual declines of FEV_1, FVC, FEV_1/FVC, and FEF_{25-75}, i.e. the differences between follow-up and baseline values of the respective parameters divided by the individual time of follow-up in years. Thus, a negative value indicates an annual loss in lung function.

2.3. Air pollution exposure and covariates

Estimates of annual average exposure to PM_{10} for the 12 months prior to assessments were assigned based on the participant's home address. They were derived from Gaussian dispersion models with predictions for the years 1990 and 2000, which were inter- and extrapolated based on continuous data from fixed air pollution monitoring stations (Liu et al., 2007). Individual change in PM_{10} exposure (ΔPM_{10}) was calculated by subtracting estimates prior to the first health assessment from the respective estimates prior to the second assessment and scaling the difference to a 10-year time span. PM_{10} exposure in all study areas declined throughout the study period.

Level of education, nationality, detailed information about current and past smoking habits, parental smoking, exposure to environmental tobacco smoke (ETS), and occupational exposure to vapors, gas, dust or fumes was collected through questionnaires. Height and weight were measured, and body mass index (BMI) was calculated as weight divided by squared height. Skin-prick tests were conducted at the baseline examination and participants were classified as having atopy if they reacted positively to one or more of the eight inhalant allergens tested (Martin et al., 1997).

2.4. Genotyping

Blood for DNA analysis was provided at the follow-up examination by participants consenting to genetic analyses (Ackermann-Liebrich et al., 2005). The lead SNPs of the ten most significantly associated regions at stage 1 of a large GWAS meta-analysis on lung function (all with $P < 8 * 10^{-8}$) were selected and chosen for replication by genotyping in SAPALDIA and other studies (Soler Artigas et al., 2011). The genotyping of the 5646 SAPALDIA subjects with complete baseline spirometry and smoking information was done using the iPLEX Gold MassARRAY (SEQUENOM, San Diego, USA). Unsuccessful genotyping occurred for varying numbers of DNA samples (rs3743563, n = 33; rs12477314, n = 39; rs11001819, n = 37; rs7068966, n = 36; rs2865531, n = 43; rs2857595, n = 22; rs2284746, n = 35; rs1551943, n = 50; rs1529672, n = 28; rs1036429, n = 33), resulting in call rates exceeding 99.1% and more for each of the ten SNPs (Supplementary Table S1).

2.5. Statistical analysis

SNPs were tested for Hardy–Weinberg-Equilibrium (HWE) using chi-square statistics. We based our statistical model on the previously published analysis identifying an attenuation of age-related lung function decline in the SAPALDIA cohort study following the reduction in PM_{10} exposure (Downs et al., 2007). Mixed linear regression models were applied to calculate genotype-specific estimates of the effect of ΔPM10 on lung function decline with 95% confidence intervals (95% CI). Reported estimates derive from multiplicative interaction terms and refer to 10 $\mu g/m^3$ declines in PM_{10} over 10 years of follow-up. The covariates included age, age squared, sex, height, parental smoking status, seasonal effects (sine and cosine function of day of examination), level of education at baseline and change in level, nationality (Swiss or other), the presence or absence of occupational exposure to vapors, gas, dust or fumes at both examinations, smoking status at follow-up (never vs. former vs. current smoking), pack-years up to and since baseline, cigarettes per day in both surveys, presence or absence of baseline atopy, PM_{10} at baseline, BMI at the first survey and change in BMI as well as the interaction between the two BMI parameters. Furthermore, we adjusted for short-term PM_{10} exposure considering the three days prior to follow-up examination. Random effects for study area were introduced to control for clustering of residuals within areas. Two-sided P-values for interaction were calculated assuming an additive genetic model, i.e. linearity of allelic effects.

In the sensitivity analysis restricted to never-smokers, the same covariates were considered except for replacing the smoking variables by exposure to ETS at baseline and during follow-up. A second sensitivity analysis replaced the annual declines of lung function by the percent change of the annual decline.

Analyses were conducted with STATA version 12.1. P-values < 0.05 were interpreted as statistically significant for main and interaction effects. Multiple testing was additionally considered using Bonferroni correction, i.e. by dividing the significance level by the number of