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Novel Genetic Susceptibility Loci for FEV_1 in the Context of Occupational Exposure in Never-Smokers

To the Editor:

Recently, we identified several novel and plausible genetic susceptibility loci for impaired lung function levels in the context of occupational exposure in a sample, including both never- and ever-smokers (1). Previous studies suggest that effects of genetic variants (2), occupational exposures (3), and their interactions (1) may be different in never-smokers and ever-smokers. Yet never-smokers generally make up a smaller proportion of subjects in general population studies (including current, former, and never-smokers), and effects solely present in never-smokers may therefore not be detected. Hence, to unravel why and how never-smokers develop impaired lung function levels and chronic respiratory diseases such as chronic obstructive pulmonary disease, it is important to study the effects of nonsmoking-related exposures without potential interference of tobacco smoke exposure. With the current genome-wide interaction study, we aimed to identify novel genetic susceptibility loci for impaired levels of FEV_1 in the context of occupational exposure to biological dust, mineral dust, and gases/fumes in a sample including never-smokers only.

We included never-smokers from two Dutch general population-based cohorts: LifeLines (N = 5,070) and Vlagtwedde-Vlaardingen (N = 431). First, in each cohort separately, genome-wide single-nucleotide polymorphism (SNP)-by-exposure interactions were assessed, using linear regression models specified as follows: FEV_1 = SNP (additive effect) + low exposure + high exposure + SNP × low exposure + SNP × high exposure + sex + age + height. To have a clear exposure contrast, we focused on the SNP-by–high exposure interaction only. Subsequently, the SNP-by–high exposure interactions from both cohorts were metaanalyzed using effects estimates weighted by the SEs. SNPs with interaction *P* values $<5 \times 10^{-8}$ and with the same direction of interaction in both cohorts were taken further for cis-acting expression quantitative trait loci (cis-eQTL) analysis in lung tissue of 1,087 subjects (4). Finally, we performed pathway analyses using all SNPs (5). More detailed information about the cohorts, phenotyping, genotyping, occupational exposure assessment, cis-eQTL, and pathway analysis can be found elsewhere (1).

Subjects included from the LifeLines study had a median age of 46 years (range, 18–90 yr), with a mean FEV_1 of 104% predicted and mean FEV_1/FVC of 78%. Subjects from the Vlagtwedde-Vlaardingen study had a median age of 54 years (range, 36–79 yr), with a mean FEV_1 of 98% predicted and mean FEV_1/FVC of 76%.

We identified four significant SNP-by–high exposure interactions, one with mineral dust and three with gases/fumes exposure (Table 1). No significant interactions were found with high exposure to biological dust. For all four SNPs, highly exposed subjects had substantially lower FEV_1 levels compared with subjects without exposure, yet only when carrying at least one copy of the risk allele and not when carrying the wild-type genotype (Figures 1A–1D). None of the four identified SNPs was a cis-eQTL in lung tissue. Finally, the Biocarta pathways patched 1 and the natural killer cells were suggestively associated (false discovery rate *P* value < 0.25) with FEV_1 in the context of mineral dust and gases/fumes exposure, respectively.

The most significant interaction identified was between gases/fumes exposure and SNP rs10223081 located nearby the gene *NMUR2*, a G coupled–protein receptor for neuromedin U (NMU) (6). NMU can induce mast cell degranulation leading to, for example, early-phase inflammation, such as neutrophil infiltration in inflamed sites (7), and can induce eosinophil infiltration in allergic inflammatory sites in an antigen-induced asthma model. We found modest expression of *NMUR2* in lung tissue (data not shown), yet this expression was not associated with the identified SNP. Importantly, effects of high exposure on gases/fumes were large and of

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Table 1. Genome-Wide Significant SNP-by–high Occupational Exposure Interactions in Association with the Level of FEV_1 in the Fixed-Effects Metaanalysis

					Metaanalysis			LifeLines *(n = 5,070)*					Vlagtwedde-Vlaardingen *(n = 431)*			
Exposure*	**SNP**	**Chr.**	**Annotation (refseq)**	**Effect allele**	***P* Value**	***b* (*ml*)**	**I^2**	***b* (*ml*)**	**95% CI**		***P* Value**	**MAF (%)**	***b* (*ml*)**	**95% CI**		***P* Value**	**MAF (%)**
Mineral dust	rs9376154	6	*PDE7B* (26kb 5′)	G	2.94E-08	−381	0	−396	−555	−237	1.02E-06	8	−342	−597	−87	8.82E-03	8
Gases/fumes	rs10223081	5	*NMUR2* (230kb 3′)	C	1.94E-09	−422	77	−386	−528	−244	1.08E-07	8	−1020	−1593	−447	5.37E-04	8
Gases/fumes	rs7548373	1	*IL10* (8kb 3′)	T	7.56E-09	−606	0	−609	−822	−397	2.06E-08	5	−555	−1349	239	1.71E-01	6
Gases/fumes	rs17092559	14	*SYNE3* (20kb 5′)	A	3.74E-08	−264	65	−245	−341	−148	7.09E-07	20	−604	−1011	−197	3.84E-03	18

Definition of abbreviations: Chr. = chromosome; CI = confidence interval; I^2 = percentage of variation across studies; MAF = minor allele frequency; SNP = single-nucleotide polymorphism. Linear regression models were adjusted for sex, age, and height. Cohort-specific effect estimates for the LifeLines and Vlagtwedde-Vlaardingen cohorts are also shown. Interaction effects (b) and corresponding 95% confidence intervals (95% CI) are given in ml FEV_1. MAF is given for the effect allele.

*Prevalence of high exposure to mineral dust is 4% for LifeLines and 18% for Vlagtwedde-Vlaardingen; prevalence of high exposure to gases/fumes is 5% for LifeLines and 4% for Vlagtwedde-Vlaardingen.

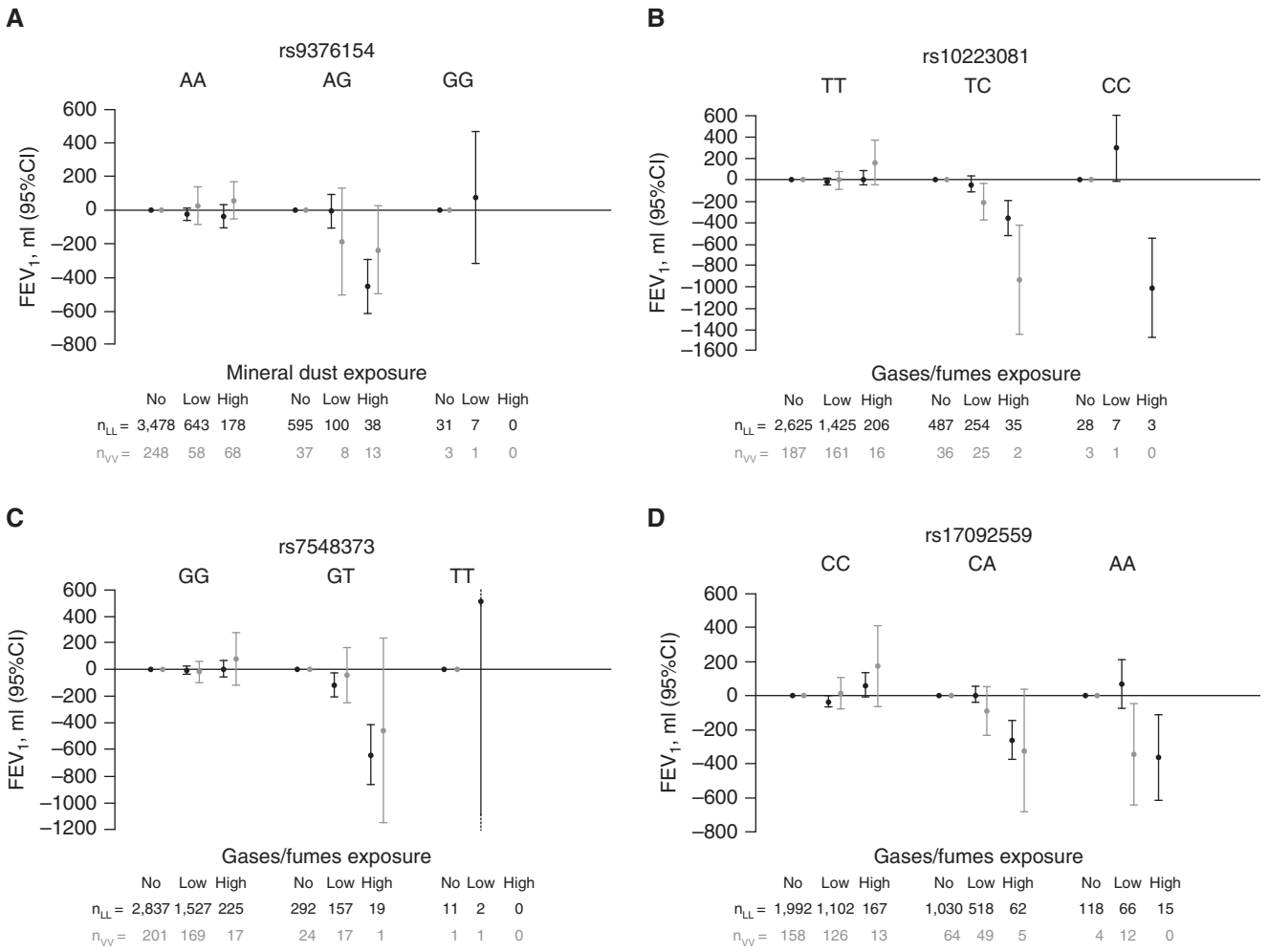

Figure 1. Associations of low and high occupational exposure (no exposure was set as reference category) with the level of FEV_1 (ml) stratified by genotype in the LifeLines cohort (*black dots*) and Vlagtwedde-Vlaardingen cohort (*gray dots*). Linear regression models were adjusted for sex, age, and height. CI = confidence interval; n_{LL} = number of subjects in each stratum in the LifeLines cohort; n_{VV} = number of subjects in each stratum in the Vlagtwedde-Vlaardingen cohort.

clinically relevant size (i.e., subjects with high exposure to gases/fumes had up to 1,000 ml lower levels of FEV_1 compared with nonexposed subjects when carrying the risk allele; such effects were not observed in subjects with wild-type genotypes).

Another interesting SNP identified was located nearby interleukin 10 (*IL10*). Although the interaction effect did not reach nominal significance in the smaller Vlagtwedde-Vlaardingen cohort, effect estimates in both cohorts were large and of similar magnitude. Findings from the current study, together with findings from previous studies, suggest IL-10 is an important anti-inflammatory mediator with protective effects on lung function after exposure to noxious gases and fumes. For example, the number of bronchoalveolar lavage fluid polymorphonuclear leukocytes and nuclear factor-κB translocations were significantly greater in *IL10*-deficient mice compared with wild types after ozone exposure (8). Further, *IL10* expression increased in bronchial epithelium of healthy subjects when they were exposed to NO_2 (9). The SNP identified in the current study was not associated with expression of *IL10* in lung tissue, yet we were, unfortunately, not able to assess gene expression after stimulation by gases and fumes. The pathway analysis suggested that natural killer cells, which produce IL-10 (10), may be involved in the development of impaired FEV_1 in the context of gases/fumes exposure, further supporting a mediating role of IL-10.

The main limitation of the current study is the small number of subjects with combinations of low-frequency genotypes and high exposure (Figure 1). However, we believe that the clear exposure–response patterns with clinically relevant effects on lung function levels, as well as the previously shown involvement of *NMU* and *IL10* in inflammatory responses, justifies further research into these genes as susceptibility loci for impaired FEV_1 in the context of gases/fumes exposure.

Of interest, none of the SNPs identified in the current study were identified in the previous study investigating ever- and never-smokers (1). In additional analyses in ever-smokers, we did not observe significant interactions ($P < 0.05$) between identified SNPs and occupational exposure (data not shown). This justifies the rationale to assess SNP-by–occupational exposure interactions in a sample of never-smokers only. Absence of interactions in

ever-smokers does not imply they are nonexistent *per se*. In fact, interactions may not be detected if both tobacco smoke and occupational exposure are acting via the same biological pathways.

To conclude, this is the first study to identify novel and biologically plausible susceptibility loci associated with lung function impairment in never-smokers. These findings provide novel insights into biological pathways underlying disease development and pinpoint novel targets for further research into lung function impairment and chronic respiratory diseases such as chronic obstructive pulmonary disease in never-smokers. ■

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Down Under in the Coal Mines

To the Editor:

We read with interest the case series by Cohen and colleagues in a recent issue of the *Journal*, describing 13 cases of U.S. coal miners with rapidly progressive massive fibrosis (1). This confirms international data reporting an increase in incidence of pneumoconiosis from several countries. In Australia, several cases of coal workers' pneumoconiosis have recently been described in the state of Queensland, as well as new cases of complicated silicosis in New South Wales. This has been the topic of much media and public interest, and a Senate Select Committee inquiry has been set up to inquire into "black lung." Previously, the perception in Australia was that pneumoconiosis had been effectively eliminated.

As representatives of the Thoracic Society of Australia and New Zealand, the peak body involved in respiratory disease in the Antipodes, we are seriously concerned about the reemergence of pneumoconiosis. It is unacceptable that new cases of pneumoconiosis should be occurring in the 21st century at a time when the knowledge regarding prevention of such a disease is excellent. Lessons learned from the past seem to have been buried in the dusts of time. Australia has no centralized reporting of occupational lung disease, and it is almost impossible to assess the annual incidence or prevalence of well-known occupational respiratory diseases. Additionally, we contend that awareness of the other lung disorders also associated with coal dust exposure is limited among medical professionals. These include interstitial pulmonary fibrosis, chronic bronchitis and emphysema, and silicosis-related lung cancer.

Respiratory science arose from the miasmas of the past, and it was the early research in the coal fields that laid the foundations of