HMOX1 and *GST* variants modify attenuation of FEF_{25–75%} decline due to PM₁₀ reduction

I. Curjuric^{*,#}, M. Imboden^{*}, C. Schindler[#], S.H. Downs[#], M. Hersberger[†],
S.L.J. Liu^{*,+}, G. Matyas[§], E.W. Russi[‡], J. Schwartz^{**}, G.A. Thun^{*}, D.S. Postma^{##},
T. Rochat^{††}, N.M. Probst-Hensch^{*} and the SAPALDIA team⁺⁺

ABSTRACT: Reduced exposure to particulate matter with a 50% cut-off aerodynamic diameter of 10 µm (PM₁₀) attenuated age-related lung function decline in our cohort, particularly in the small airways. We hypothesised that polymorphisms in glutathione S-transferase (*GST*) and haem oxygenase-1 (*HMOX1*) genes, important for oxidative stress defence, modify these beneficial effects.

A population-based sample of 4,365 adults was followed up after 11 yrs, including questionnaire, spirometry and DNA blood sampling. PM₁₀ exposure was estimated by dispersion modelling and temporal interpolation. The main effects on annual decline in forced expiratory flow at 25–75% of forced vital capacity (FEF_{25–75%}) and interactions with PM₁₀ reduction were investigated for polymorphisms *HMOX1* rs2071746 (T/A), rs735266 (T/A) and rs5995098 (G/C), *HMOX1* (GT)_n promoter repeat, *GSTM1* and *GSTT1* deletions, and *GSTP1* p.Ile105Val, using mixed linear regression models.

HMOX1 rs5995098, *HMOX1* haplotype TTG and *GSTP1* showed significant genetic main effects. Interactions with PM₁₀ reduction were detected: a 10 µg·m⁻³ reduction significantly attenuated annual FEF_{25–75%} decline by 15.3 mL·s⁻¹ only in the absence of *HMOX1* haplotype ATC. Similarly, carriers of long (GT)_n promoter repeat alleles or the *GSTP1* Val/Val genotype profited significantly more from a 10 µg·m⁻³ reduction (26.5 mL·s⁻¹ and 27.3 mL·s⁻¹ respectively) than non-carriers.

Benefits of a reduction in PM₁₀ exposure are not equally distributed across the population but are modified by the individual genetic make-up determining oxidative stress defence.

KEYWORDS: Exposure to particles with a 50% cut-off aerodynamic diameter of 10 µm, forced expiratory flow at 25–75% of forced vital capacity, general population sample, glutathione S-transferase, haem oxygenase-1, lung function decline

Several studies have shown deleterious effects of air pollution on lung function, including our own [1–3]. The underlying inflammatory reactions, triggered by free radicals present in or induced by inhaled dusts, fumes, tobacco smoke and environmental air pollution [4], lead to adverse health effects in the long term [5]. Exposure to radicals is termed “oxidative stress”, and its contribution to lung diseases supports the hypothesis that polymorphisms in genes involved in oxidative stress defence importantly determine susceptibility to external noxious exposures. Polymorphisms of the haem oxygenase-1 (*HMOX1*) gene and glutathione S-transferase (*GST*)

super-gene family are interesting candidates in this respect.

The *HMOX1* gene is rapidly upregulated in the alveolar cell layer after exposure to oxidative stress and forms part of the lung’s first line of defence [6]. The enzyme catalyses haem degradation, producing bilirubin, CO and ferritin, which exert important antioxidative and anti-inflammatory effects [6]. A microsatellite polymorphism, *i.e.* a genetic variant consisting of a variable number of short nucleotide sequence repetitions (in this case “GT”), is known in the promoter region of the *HMOX1* gene. In general population samples from

AFFILIATIONS

^{*}Chronic Disease Epidemiology, Institute of Social and Preventive Medicine, University of Zürich,

[†]Division of Clinical Chemistry and Biochemistry, University Children’s Hospital Zürich,

[‡]Institute of Medical Genetics, Division of Medical Molecular Genetics and Gene Diagnostics, University of Zürich,

[§]Division of Pulmonary Medicine, University Hospital of Zürich, Zürich,

^{||}Institute of Social and Preventive Medicine, University of Basel, Basel,

[¶]Division of Pulmonary Medicine, University Hospitals of Geneva, Geneva, Switzerland,

[‡]Dept of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA,

^{**}Dept of Environmental Health, Harvard School of Public Health, Boston, MA, USA,

^{##}Dept of Pulmonology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, and

⁺⁺Members of the SAPALDIA team are listed in the Acknowledgements section.

CORRESPONDENCE

N.M. Probst-Hensch, Dept of Chronic Disease Epidemiology, Institute of Social and Preventive Medicine, Swiss Tropical Institute Basel, Steinengraben 49, CH-4051 Basel, Switzerland
E-mail: Nicole.Probst@unibas.ch

Received:

March 18 2009

Accepted after revision:

Aug 24 2009

First published online:

Sept 09 2009

European Respiratory Journal

Print ISSN 0903-1936

Online ISSN 1399-3003

This article has supplementary material available from www.erj.ersjournals.com

France and the Netherlands, long repeat alleles (≥ 33 repeats) were associated with accelerated decline in several lung function parameters, especially among smokers [7, 8].

The *GST* super-gene family comprises various isoforms, including *GSTT1*, *GSTM1* and *GSTP1* [9]. GSTs are phase II biotransformation enzymes and important antioxidants. Their substrate, glutathione (GSH), is abundant in the alveolar fluid line and its synthesis is upregulated upon oxidative stress [10]. A single nucleotide polymorphism (SNP) in *GSTP1*, i.e. Ile105Val leads to the substitution of isoleucine (Ile) for valine (Val) in protein synthesis and alters the detoxification of diolepoxides [11]. In *GSTT1* and *GSTM1*, homozygous gene deletions with complete loss of protein function are highly prevalent [9]. Genetic variation in *GSTT1*, *GSTM1* and *GSTP1* has been associated with accelerated lung function decline in the adult general population [12] and reduced lung function growth in children [13].

The Swiss Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) recently showed that a reduction in long-term exposure to particulate matter with a 50% cut-off aerodynamic diameter of 10 μm (PM₁₀) was associated with a significant attenuation of the age-related decline in lung function over 11 yrs of follow-up [2]. Attenuations were strongest for forced expiratory flow at 25–75% of forced vital capacity (FEF_{25–75%}), and weakened with increasing baseline PM₁₀ exposure. In the present study we thus focused on the decline in FEF_{25–75%} and investigated whether the effect of PM₁₀ reduction differed according to the participants' genetic make-up regarding *HMOX1*, *GSTM1*, *GSTT1* and *GSTP1* genes, which all play a major role in the oxidative stress defence of the body.

METHODS

Study design and population

The methods of the SAPALDIA study have been described in detail elsewhere [14]. In short, the study population consists of a random population sample of adults aged 18–60 yrs from eight areas of Switzerland. A total of 9,651 people participated in the first assessment in 1991, and at the follow-up examination in 2002, 8,047 were reassessed. Written consent was obtained from all study participants. Approval of the study was given by the Swiss Academy of Medical Sciences (Basel, Switzerland) and the regional ethics committees.

The present study sample consists of 4,365 participants with complete data regarding spirometry, residential history, smoking history, PM₁₀ exposure and available genotyping information (fig. 1).

Assessment procedures

Health questionnaire

At both surveys, participants underwent a computer-assisted interview comprising questions about smoking behaviour, exposure to environmental tobacco smoke at home and at the workplace, workplace exposure to dust and fumes, presence of respiratory symptoms or chronic illness, medication use and socio-economic factors. Never-smokers were defined as people who, at the time of follow-up, had smoked fewer than 20 packs of cigarettes or 360 g of tobacco during their lifetime, and smokers and ex-smokers reported current smoking or quitting at least 1 month before follow-up, respectively. For smokers

FIGURE 1. Selection of study participants. PM₁₀: particulate matter with a 50% cut-off aerodynamic diameter of 10 μm .

and ex-smokers, smoking history in pack-years before the first assessment and between surveys were included in the analyses.

Pulmonary function testing

Spirometry testing was performed according to the European Community Respiratory Health Survey protocol [15] and complied with American Thoracic Society criteria [16]. Device checks and calibrations were performed on a daily basis during assessment periods. The same spirometric devices were used at both examinations (Sensor Medics 2200 SP; Sensor Medics, Yorba Linda, CA, USA). Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁) and FEF_{25–75%} were recorded. Annual decline in FEF_{25–75%} was calculated by subtracting the baseline measurement from the follow-up value and dividing the difference by the individual time of follow-up in years.

Atopy testing

In 1991, skin-prick testing was performed for cat fur and dog epithelia, timothy grass, *Parietaria*, birch, house-dust mite, *Alternaria tenuis* and *Cladosporium herbarum*. A person was considered atopic if the mean diameter of at least one wheal was 3 mm greater than that of a control sting free of antigen

[17, 18]. The mean diameter was calculated by averaging the sum of the widest diameter and the longest line perpendicular to it within the wheal perimeter.

Genotyping procedures

A detailed description of the genotyping procedures and specific genetic terms is given in the online supplementary document.

Briefly, genomic DNA was extracted manually from EDTA-buffered whole blood.

Genotyping of *GSTT1*, *GSTM1* gene deletions and *GSTP1* p.Ile105Val SNP in the SAPALDIA cohort have been previously reported [12].

Three SNPs of the *HMOX1* gene, rs2071746 (T/A), rs5995098 (G/C) and rs735266 (T/A) were genotyped using real-time PCR (TaqMan®; Applied Biosystems, Rotkreuz, Switzerland). *HMOX1* haplotypes were inferred using PHASE software v2.1 [19, 20]. Haplotypes with prevalences below 5% were not analysed.

HMOX1 (GT)_n promoter repeats were determined using PCR and fragment size analysis with GeneMapper® software v3.5 (Applied Biosystems). For comparability with previous studies showing associations of ≥33 repeats with lung function [7, 8], repeat genotypes were classified as having at least one long allele (≥33 repeats) or not. The allele distribution in the study population is presented in figure 2.

Air pollution exposure

The attribution of PM₁₀ exposure has been described in detail previously [2, 21]. Each participant was assigned annual average PM₁₀ concentrations for 1991 and 2002 based on their home address using a Gaussian dispersion model (PolluMap version 2.0 [22]) with predictions for the years 1990 and 2000, and an algorithm interpolating historical trends in central site measurements. Individual annual change in PM₁₀ exposure, our exposure of interest, was calculated by subtracting 1991 values from those in 2002 and dividing the result by the number of years of follow-up. PM₁₀ exposure in Switzerland declined throughout the study period, therefore the median change in PM₁₀ exposure was negative.

FIGURE 2. Distribution of (GT)_n repeats of the *HMOX1* promoter polymorphism.

Statistical analysis

A detailed characterisation of the study population can be found in online table O1. To address the influence of self-selected participation, our study sample was compared with all participants with complete data at the baseline examination, but not at follow-up.

The main effects of alleles on annual decline in FEF_{25–75%} were assessed using multivariable linear regression with random effects for study areas under specification of additive, dominant and recessive genetic models (“additive” meaning a trend with the number of mutant alleles, “dominant” effects in heterozygous and homozygous and “recessive” only in homozygous mutant alleles). In accordance with DOWNS *et al.* [2], all models included the average annual PM₁₀ exposure at baseline and its annual change, and adjusted for sex, age, age squared, height and atopy as baseline variables, smoking status (current smoker, former smoker, never-smoker) at follow-up, smoking intensity (number of cigarettes per day) at both surveys, smoking history in pack-years up to the baseline and between surveys as cumulative smoking exposure, parental smoking, workplace exposure to dust and fumes at each survey, body mass index (BMI) and its change (baseline BMI, change in BMI and an interaction term between the two), level of education and its change, nationality (Swiss and non-Swiss) and seasonal terms (sine and cosine functions of the day of examination).

Interaction between PM₁₀ decline and genotypes was tested by recoding all polymorphisms into categorical variables representing dominant, co-dominant and recessive effects and including genetic variables and their products with change in PM₁₀ into the regression models. Reparametrisation of annual PM₁₀ decline into genotype-specific variables yielded genotype-specific PM₁₀ effects and 95% confidence intervals. Reported effects refer to a 10 µg·m⁻³ decline in PM₁₀ over the average follow-up period of 10.92 yrs. To control for the influence of FVC on the measurement of FEF_{25–75%} [23], the analysis was repeated after adjusting FEF_{25–75%} values at baseline and follow-up for FVC *via* linear regression and recalculating change in FEF_{25–75%} using the residuals.

Separate analyses of main effects and interactions were done for males and females.

In a sensitivity analysis, the study sample was stratified into never- and ever-smokers (being a current smoker or ex-smoker at baseline or follow-up) to check whether the findings were stable across smoking categories. PM₁₀ effects within genotype strata were tested for effect modification by ever-smoking.

To assess a potential multiple testing problem, we subjected all significant gene-air pollution interactions to permutation testing using STATA’s “permute” procedure with 10,000 runs. This test permutes the outcome variable and rematches to the genotype data, creating new data sets where null associations are expected. The p-values for gene-air pollution interactions are computed as the fraction of permuted tests that were more significant than the original one.

Statistical analyses were performed using SAS software version 9.1 (SAS Institute, Cary, NC, USA) and STATA version 9.2 (StataCorp, College Station, TX, USA). Significance levels

for two-sided tests of main effects were chosen at $\alpha=0.05$, and at $\alpha=0.1$ for tests of effect modification.

RESULTS

Characteristics of study participants

Characteristics of participants included in the present study are presented in table 1. At baseline, 52.9% of our study sample were female, the mean age was 41.4 yrs, and 29.1% of participants were smokers with a median of 18.4 pack-yrs smoked. Mean values for FVC at the baseline examination were 4.5 L, for FEV1 3.6 L and for FEF25–75% 3.4 L·s⁻¹. A more detailed characterisation can be found in online table O1.

When compared with individuals who completed baseline assessment but were not included in this study for reasons described in figure 1 (see Methods section), our study sample was slightly older, leaner and showed a higher percentage of never-smokers, had better education and slightly higher lung function values.

Main effects of gene polymorphisms

Estimates of gene main effects on change in FEF25–75% during follow-up in the whole study sample are presented in table 2, sex-specific results are shown in online table O2.

TABLE 1

Characteristics of study sample and excluded participants

Variable	Baseline participants not included in the current study [#]	Current study sample [†]	p-values [‡]
Females %	50.2	52.9	0.010
Age in 1991 yrs	40.7 ± 12.0	41.4 ± 11.3	0.005
Being a smoker in 1991 %	37.6	29.1	<0.001
Being a never-smoker in 1991 %	39.7	48.2	<0.001
Being a smoker in 2002 %	NA	21.8	
Being a never-smoker in 2002 %	NA	46.9	
Pack-yrs in smokers 1991	17.7 (7.3–31.7)	18.4 (8.0–31.8)	0.470
Pack-yrs in smokers 2002	NA	21.6 (8.6–35.9)	
High educational level in 1991 %	15.8	17.3	0.058
FVC in 1991 L	4.4 ± 1.0	4.5 ± 1.0	<0.001
Change in FVC up to 2002 L		-0.3 ± 0.4	
FEV1 in 1991 L	3.5 ± 0.9	3.6 ± 0.8	0.004
Change in FEV1 up to 2002 L		-0.4 ± 0.3	
FEV1/FVC ratio >0.7 in 1991 %	86.9	89.8	<0.001
FEF25–75% in 1991 L·s ⁻¹	3.4 ± 1.3	3.4 ± 1.2	0.952
Change in FEF25–75% up to 2002 L·s ⁻¹		-0.8 ± 0.7	
Change in PM10 exposure during follow-up µg·m ⁻³	NA	-5.3 (-7.3– -4.2)	

Data are presented as mean ± SD or median (interquartile range), unless stated otherwise. [#]: 4,685 subjects (sample size may decrease to 4,517 due to missing values); [†]: 4,365 subjects; [‡]: included versus not included, significant p-values are presented in bold. FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; FEF25–75%: forced expiratory flow at 25–75% of FVC; PM10: particulate matter with a 50% cut-off aerodynamic diameter of 10 µm.

HMOX1 polymorphisms

The homozygous mutant SNP alleles of all three *HMOX1* SNPs and related haplotypes showed mostly favourable effects, attenuating the natural decline in FEF25–75% in the whole study sample by up to 5.9 mL·s⁻¹·yr⁻¹ (95% CI -0.5–12.3). Associations were statistically significant for *HMOX1* rs5995098 (p=0.047) and *HMOX1* haplotype TTG (p=0.031) under a recessive genetic model.

No significant association with FEF25–75% decline was found for having at least one long (GT)_n promoter repeat allele.

GST polymorphisms

GSTP1 was associated with annual FEF25–75% decline (p=0.044) under a dominant genetic model, and the effect was marginally significant under an additive model (p=0.054): valine alleles accelerated decline by up to 4.4 mL·s⁻¹ (table 2).

Interaction of genetic polymorphisms with reduction in PM10 exposure on lung function decline

Interactions of gene polymorphisms and decline in PM10 exposure in the whole study population on FEF25–75% decline are presented in table 3. Sex-specific results are presented in online tables O3 and O4. Analyses equivalent to the ones for FEF25–75% were done for change in FEV1 and FVC, but only weak interaction signals were found (online table O5).

HMOX1 polymorphisms

In the whole study sample, no significant interaction with decline in PM10 exposure was found for single *HMOX1* SNPs, but rather for haplotypes: in participants not carrying haplotype ATC a PM10 decline of 10 µg·m⁻³ during follow-up was significantly associated with an attenuation of the annual decline in FEF25–75% by 15.3 mL·s⁻¹ (95% CI 7.8–22.7), opposed to no significant effects in haplotype ATC carriers (table 3). The p-value for interaction was 0.009 when testing a dominant genetic model. There was also an interaction between decline in PM10 and having at least one long *HMOX1* (GT)_n promoter repeat allele: participants with long repeat alleles presented an attenuation of the annual FEF25–75% decline by 26.5 mL·s⁻¹ (95% CI 11.7–41.2), as opposed to only 11.7 mL·s⁻¹ (95% CI 4.3–19.2) in those without long repeat alleles. This interaction was statistically significant (p=0.044).

GST polymorphisms

No significant interactions between *GSTM1* or *GSTT1* deletions and PM10 exposure reduction were found.

Regarding the *GSTP1* p.Ile105Val polymorphism, participants with genotype Val/Val showed the most beneficial reaction to PM10 reduction with an attenuation of the decline in FEF25–75% by 27.3 mL·s⁻¹ (95% CI 11.4–43.2). Those with one or no Val allele presented attenuations of 9.8 mL·s⁻¹ (95% CI 0.7–19.0) and 13.5 mL·s⁻¹ (95% CI 5.2–21.8), respectively. Under a recessive genetic model, this interaction was statistically significant (p=0.052).

Reanalysis after adjustment for FVC values

Repeating the analysis using FEF25–75% adjusted for FVC to calculate change in FEF25–75% did not yield different results.

TABLE 2 Main effects of haem oxygenase-1 (*HMOX1*) and glutathione S-transferase (*GST*) polymorphisms on change in forced expiratory flow at 25–75% of forced vital capacity (FEF_{25–75%})

Gene polymorphism	Subjects n	Gene main effect on decline in FEF _{25–75%} [#] mL·s ⁻¹ ·yr ⁻¹	95% CI	p-value
<i>HMOX1</i> SNPs				
<i>HMOX1</i> rs2071746				
AA	1401	Ref.		
AT	2089	0.8	-3.3–4.9	0.053 ^f
TT	763	5.1	-0.2–10.5	
<i>HMOX1</i> rs735266				
AA	1669	Ref.		
AT	2022	1.2	-2.7–5.1	0.128 ^{##}
TT	595	4.7	-1.0–10.4	
<i>HMOX1</i> rs5995098				
CC	2021	Ref.		
CG	1876	-1.6	-5.4–2.2	0.047^f
GG	427	5.3	-1.0–11.7	
<i>HMOX1</i> haplotypes[‡]				
Haplotype AAC				
0 alleles	798	Ref.		
1 allele	2178	-3.3	-8.2–1.6	0.09 ^{##}
2 alleles	1389	-4.7	-10.0–0.5	
Haplotype ATC				
0 alleles	3925	Ref.		
1 allele	431	2.0	-4.0–8.1	0.476 ^{##}
2 alleles	9	5.6	-33.9–45.1	
Haplotype TTC				
0 alleles	3873	Ref.		
1 allele	478	4.3	-1.5–10.0	0.156 ^{†¶}
2 alleles	14	-2.1	-33.8–29.6	
Haplotype TTG				
0 alleles	2057	Ref.		
1 allele	1895	-1.7	-5.5–2.1	0.031^f
2 alleles	413	5.9	-0.5–12.3	
<i>HMOX1</i> promoter repeat polymorphism				
<i>HMOX1</i> (GT) _n long allele ⁺				
0 alleles	3919	Ref.		
1 or 2 alleles	446	-1.1	-7.0–4.9	0.728
<i>GST</i> polymorphisms				
<i>GSTM1</i> deletion [§]				
No	2032	Ref.		
Yes	2319	-1.1	-4.7–2.5	0.558
<i>GSTT1</i> deletion [§]				
No	3554	Ref.		
Yes	797	-1.1	-5.8–3.6	0.644
<i>GSTP1</i> Val/Ile polymorphism				
Ile/Ile	2061	Ref.		
Ile/Val	1892	-3.6	-7.3–0.2	0.044^{†¶}
Val/Val	401	-4.4	-10.9–2.1	

SNP: single nucleotide polymorphism. [#]: negative values represent accelerations of the natural decline, positive values attenuations compared with the reference group. Estimates from a multivariable linear regression of annual change in FEF_{25–75%} on genetic variants adjusted for particulate matter with a 50% cut-off aerodynamic diameter of 10 µm (PM₁₀) exposure at baseline; annual change in PM₁₀; sex, age, age squared, height and atopy at baseline; smoking status at follow-up; cigarettes per day in smokers at both surveys; pack-years up to baseline and between surveys; parental smoking; workplace exposure to dust and fumes at each survey; baseline body mass index (BMI); change in BMI and the interaction between the two; level of education and its change; nationality; season of assessment and clustering within area. [†]: determined from rs2071746, rs735266 and rs5995098 SNPs (in this sequence) using PHASE software v2.1 [19, 20]. ⁺: *HMOX1* (GT)_n long allele is defined as having at least 33 GT repeats. [§]: deletions are complete homozygous gene deletions (null versus non-null). ^f: under a recessive genetic model; ^{##}: under an additive genetic model; ^{††}: under a dominant genetic model. Significant p-values are presented in bold.

TABLE 3 Effect of a decrease in exposure to particles with a 50% cut-off aerodynamic diameter of 10 µm (PM₁₀) on change in forced expiratory flow at 25–75% of forced vital capacity (FEF_{25–75%}), by haem oxygenase-1 (*HMOX1*) and glutathione S-transferase (*GST*) genotypes

Gene polymorphism	Subjects n	Effect of a 10 µg·m ⁻³ decrease in PM ₁₀ over 10.92 yrs (mean follow-up) on			
		Annual change in FEF _{25–75%} [#] mL·s ⁻¹ ·yr ⁻¹		Annual change in FEF _{25–75%} mL·s ⁻¹ ·yr ⁻¹ adjusted for FVC ^{#,†}	
		Estimate	95% CI	Estimate	95% CI
HMOX1 SNPs					
<i>HMOX</i> rs2071746					
AA	1401	14.2	4.9 to 23.6	14.9	4.9 to 24.8
AT	2089	14.2	5.3 to 23.2	15.9	6.5 to 25.4
TT	763	10.8	-1.5 to 23.1	12.9	-0.1 to 26.0
<i>HMOX</i> rs735266					
AA	1669	10.7	1.9 to 19.4	11.4	2.0 to 20.7
AT	2022	17.5	8.4 to 26.7	19.5	9.8 to 29.2
TT	595	11.9	-1.4 to 25.2	14.6	0.5 to 28.7
<i>HMOX</i> rs5995098					
CC	2021	11.0	2.7 to 19.3	11.6	2.8 to 20.4
CG	1876	17.5	8.2 to 26.8	19.3	9.4 to 29.1
GG	427	10.8	-4.8 to 26.5	12.8	-3.9 to 29.4
HMOX1 haplotypes⁺					
Haplotype AAC					
0 alleles	798	10.6	-1.5 to 22.8	12.5	-0.4 to 25.4
1 alleles	2178	14.2	5.5 to 22.9	15.7	6.5 to 25.0
2 alleles	1389	13.1	3.9 to 22.3	13.5	3.6 to 23.3
Haplotype ATC					
0 alleles	3925	15.3^{##}	7.8 to 22.7	16.5^{††}	8.6 to 24.4
1 alleles	431	-4.3	-18.9 to 10.4	-3.3	-18.9 to 12.3
2 alleles	9	31.9	-62.3 to 126.1	8.8	-91.3 to 109.0
Haplotype TTC					
0 alleles	3873	13.0	5.6 to 20.5	14.0	6.1 to 21.9
1 alleles	478	11.1	-3.9 to 26.0	14.0	-2.0 to 29.9
2 alleles	14	36.5	-9.8 to 82.8	36.9	-12.4 to 86.1
Haplotype TTG					
0 alleles	2057	11.2	3.0 to 19.5	11.9	3.1 to 20.6
1 alleles	1895	17.2	7.9 to 26.5	18.9	9.0 to 28.7
2 alleles	413	10.7	-5.0 to 26.3	12.6	-4.0 to 29.2
HMOX1 promoter repeat polymorphism					
<i>HMOX</i> (GT) _n long allele [§]					
0 alleles	3919	11.7	4.3 to 19.2	12.8	4.9 to 20.7
1 or 2 alleles	446	26.5⁺⁺	11.7 to 41.2	27.5^{§§}	11.8 to 43.2
GST polymorphisms					
<i>GSTM1</i> deletion [‡]					
No	2032	13.2	4.4 to 22.0	13.7	4.4 to 23.0
Yes	2319	13.3	5.1 to 21.6	14.9	6.1 to 23.6
<i>GSTT1</i> deletion [‡]					
No	3554	14.4	6.8 to 22.0	15.3	7.2 to 23.3
Yes	797	9.1	-2.6 to 20.8	10.8	-1.7 to 23.2
<i>GSTP1</i> Val/Ile polymorphism					
Ile/Ile	2061	13.5	5.2 to 21.8	14.3	5.4 to 23.1
Ile/Val	1892	9.8	0.7 to 19.0	11.8	2.0 to 21.5
Val/Val	401	27.3^{‡‡}	11.4 to 43.2	26.7^{###}	9.8 to 43.7

FVC: forced vital capacity; SNP: single nucleotide polymorphism. [#]: positive values represent attenuations of annual natural decline in FEF_{25–75%}, compared with a reference group experiencing no air pollution reduction. Estimates from a multivariable linear regression of annual change in FEF_{25–75%} on genetic variants, change in PM₁₀ and their interaction adjusted for PM₁₀ exposure at baseline; sex, age, age squared, height and atopy at baseline; smoking status at follow-up; cigarettes per day in (continued on next page)

TABLE 3 Continued.

smokers at both surveys; pack-years up to baseline and between surveys; parental smoking; workplace exposure to dust and fumes at each survey; baseline body mass index (BMI); change in BMI and the interaction between the two; level of education and its change; nationality; season of assessment and clustering within area. *: adjustment for FVC was done by regressing FEF_{25-75%} on FVC and using residuals for calculation of the change. †: determined from rs2071746, rs735266 and rs5995098 single nucleotide polymorphisms (in this sequence) using PHASE software v2.1 [19, 20]. ‡: HMOX1 (GT)_n long allele is defined as having at least 33 GT repeats. §: deletion means complete homozygous gene deletion (null versus non-null). ##: $p_{\text{interaction}}=0.009$ under dominant genetic model; ††: $p_{\text{interaction}}=0.011$ under dominant genetic model; †††: $p_{\text{interaction}}=0.044$; §§: $p_{\text{interaction}}=0.06$; §§§: $p_{\text{interaction}}=0.052$ under recessive genetic model; §§§§: $p_{\text{interaction}}=0.107$ under recessive genetic model. Significant p-values are presented in bold.

Apart from small changes in effect size, the patterns of interaction persisted (table 3).

Sex-specific interactions between genetic polymorphisms and PM₁₀ decline

The sex-specific results suggested that the interaction of PM₁₀-decline with HMOX1 haplotype ATC was accentuated in males (fig. 3), while that with long HMOX1 (GT)_n promoter repeat alleles was only seen in females. Genotype-specific PM₁₀-effects are presented in online tables O3 and O4.

Sensitivity analysis

Stratification and statistical interaction testing showed that the interaction effects between PM₁₀ decline and HMOX1 and GST polymorphisms did not differ between never- and ever-smokers (data not shown).

Assessment of multiple testing

The adjusted p-values for the interaction between PM₁₀ decline and HMOX1 haplotype ATC were $p=0.009$ (dominant genetic model), $p=0.041$ for a long HMOX1 (GT)_n promoter repeat and

FIGURE 3. Estimated attenuation of the annual rate of decline in forced expiratory flow at 25–75% of forced vital capacity (FEF_{25-75%}) associated with a 10 µg·m⁻³ decline in exposure to particulate matter with a 50% cut-off aerodynamic diameter of 10 µm (PM₁₀) over 11 yrs of follow-up, in a) males and b) females, stratified by genotype. Positive values indicate attenuation of decline in FEF_{25-75%}. #: $p_{\text{interaction}}=0.008$ assuming dominant genetic effects; †: $p_{\text{interaction}}=0.007$ assuming dominant genetic effects; ††: $p_{\text{interaction}}=0.028$; §: $p_{\text{interaction}}=0.052$ assuming recessive genetic effects; †: long allele is ≥ 33 GT promoter repeats.

$p=0.049$ for *GSTP1* Val/Val genotype (recessive genetic model).

DISCUSSION

The SAPALDIA study previously showed that an improvement in long-term PM₁₀ exposure was associated with an important attenuation in age-related lung function decline [2].

In the current work we demonstrate that polymorphisms in *GST* and *HMOX1* affect the age-related decline in FEF_{25–75%} *per se*, but their impact is especially pronounced in modifying the effect of reduced PM₁₀ exposure during the time of follow-up. Participants with mutant alleles in the *HMOX1* SNPs or haplotypes, with long *HMOX1* (GT)_n promoter repeat alleles or with *GSTP1* valine homozygosity, profited most from the improvement in air quality. For FEV₁ decline, a similar but weaker signal was detected in *GSTP1* valine homozygous participants. For *HMOX1* haplotype ATC, effects on FEV₁ and FVC decline appeared unstable, despite marginally significant interactions. The emphasised effect on FEF_{25–75%} decline may be due to preferential deposition of the fine, more health-relevant portion of PM₁₀ (PM_{2.5} and smaller) in smaller airways [24], and is not unexpected, as PM₁₀ reduction affected FEF_{25–75%} decline most strongly in our previous study [2].

The main effects and interactions with PM₁₀ reduction differed by polymorphism in *HMOX1*. The *GSTP1* SNP exhibited main and PM₁₀ modifying effects. The *GSTP1* main effect was most significant under a dominant ($p=0.044$) and marginally significant under an additive genetic model, while the interaction was strongest under a recessive model ($p=0.052$). This discrepancy is in line with findings from the few studies to date that have assessed genetic susceptibility to air pollutants, and all included *GSTP1*. The effect of the *GSTP1* alleles was dependent on the chosen outcome and exposure: the homozygous *GSTP1* isoleucine allele was associated with asthma in two childhood studies only in the presence of high urban background air pollution or ozone exposure [25, 26]. In contrast, *GSTP1* valine alleles were associated with respiratory symptoms in children highly exposed to ozone and with allergic sensitisation in adults exposed to traffic air pollution [27, 28]. Experimental studies in ragweed-sensitised adults showed stronger inflammatory reactions upon allergen exposure for *GSTM1*-null or *GSTP1* 105Ile genotypes only in the co-presence of diesel exhaust or environmental tobacco smoke [29]. These studies further confirm the importance of gene-environment interactions in the development of respiratory disease.

Our study benefits from a large population-based sample with detailed information on health parameters and validated exposure to air pollution (PM₁₀) on an individual scale, as well as high-quality data on genetic variants. Limitations of our study include a possible selection of healthier individuals, as indicated by the figures in table 1. However, the mean baseline value of FEF_{25–75%} did not differ between our study sample and the cohort subjects not included. Moreover, participation is unlikely to be influenced by the genetic profile of the individual, and any residual factors clustering at the area level are captured by random effects. With respect to the lower proportion of smokers in the study sample, our sensitivity

analysis showed that our main findings were also present in participants with past or current smoking exposure. Our study determined change in lung function values using only two spirometry measurements, which is a limitation. However, we expect the measurement error to be randomly distributed across the investigated genetic variants. The observed associations are therefore rather underestimated.

Another concern is population stratification. No deviance from Hardy-Weinberg equilibrium was detected for bi-allelic polymorphisms in our study sample, and the proportion of *HMOX1* long promoter repeat alleles did not significantly differ across study and language areas. Lack of suitable genetic markers prevented the application of genomic control methods [30], but since genetic homogeneity of Caucasian Western-Central European populations has been described [31], we do not expect population stratification to invalidate our findings.

We have not comprehensively assessed the genetic variation in *HMOX1*, and the functional impact of the *HMOX1* SNPs and corresponding haplotypes is still unknown. Thus, our results must be confirmed upon availability of functionally well-characterised *HMOX1* genetic variants.

Finally, it was beyond the scope of this current study to investigate how modifiable lifestyle factors like diet, physical activity or comorbidities determine susceptibility to air pollution. This will be an important aspect for future studies including our own.

In conclusion, our results show that a reduction in long-term exposure to PM₁₀ does not exert uniform beneficial effects for all members of a population. They rather indicate that individuals who differ in coping with oxidative stress due to genetic variation in *HMOX1* and *GST* enzymes also differ in their response to air quality improvements. Genetic susceptibility must be considered in setting limits for air pollution levels, which should protect the most susceptible members of society [32].

SUPPORT STATEMENT

Support was provided by the Swiss National Science Foundation (Berne, Switzerland) (grants 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 32-65896.01, 32-59302.99, 32-52720.97, 32-4253.94, 4026-28099), the Federal Office for Forest, Environment and Landscape (Berne), the Federal Office of Public Health (Berne), the Federal Office of Roads and Transport (Berne), the canton governments of Aargau, Basel Stadt, Basel-Land, Geneva, Luzern, Ticino and Zurich, the Swiss Lung League (Berne), the canton Lung League of Basel Stadt/Basel Landschaft, Geneva, Ticino and Zurich, and UBS Wealth Foundation (Zurich, Switzerland) grant BA29s8Q7-DZZ.

STATEMENT OF INTEREST

A statement of interest for D.S. Postma can be found at www.erj.ersjournals.com/misc/statements.dtl

ACKNOWLEDGEMENTS

SAPALDIA team members are as follows.

Study directorate: T. Rochat (pneumology; Geneva, Switzerland), U. Ackermann-Lieblich (epidemiology; Basel, Switzerland), J.M. Gaspoz (cardiology; Geneva), P. Leuenberger (pneumology; Lausanne,

Switzerland), L.J.S. Liu (exposure; Basel), N.M. Probst Hensch (epidemiology, and genetic and molecular biology; Zurich, Switzerland) and C. Schindler (statistics; Basel).

Scientific team: J.C. Barthélémy (cardiology; St Etienne, France), W. Berger (genetic and molecular biology; Schwerzenbach, Zurich), R. Bettschart (pneumology; Aarau, Switzerland), A. Bircher (allergology; Basel), G. Bolognini (pneumology; Lugano, Switzerland), O. Bröndli (pneumology; Wald, Switzerland), M. Brutsche (pneumology; St Gallen, Switzerland), L. Burdet (pneumology; Payerne, Switzerland), M. Frey (pneumology; Aarau), M.W. Gerbase (pneumology; Geneva), D. Gold (pneumology, epidemiology and cardiology; Basel), W. Karrer (pneumology; Montana, Switzerland), R. Keller (pneumology; Aarau), B. Knöpfli (pneumology; Berne, Switzerland), N. Künzli (epidemiology and exposure; Basel), U. Neu (exposure; Berne), L.P. Nicod (pneumology; Lausanne), M. Pons (pneumology; Lugano), E.W. Russi (pneumology; Zurich, Switzerland), P. Schmid-Grendelmeyer (allergology; Zurich), J. Schwartz (epidemiology; Boston, MA, USA), P. Straehl (exposure; Berne), J.M. Tschopp (pneumology; Montana), A. von Eckardstein (clinical chemistry; Zurich), J.P. Zellweger (pneumology; Lausanne) and E. Zemp Stutz (epidemiology; Basel).

Scientific team at coordinating centres: P.O. Bridevaux (pneumology; Geneva), I. Curjuric (epidemiology Zurich), J. Dratva (epidemiology; Basel), D. Felber Dietrich (cardiology; Basel), A. Gemperli (statistics; Basel), D. Keidel (statistics; Basel), M. Imboden (genetic and molecular biology; Zurich), H. Phuleria (exposure; Basel) and G.A. Thun (genetic and molecular biology; Zurich).

The study could not have been done without the help of the study participants, technical and administrative support and the medical teams and field workers at the local study sites.

Local fieldworkers: Aarau: M. Broglie, M. Bünter and D. Gashi; Basel: R. Armbruster, T. Damm, U. Egermann, M. Gut, L. Maier, A. Vögelin and L. Walter; Davos: D. Jud and N. Lutz; Geneva: M. Ares, M. Bennour, B. Galobardes and E. Namer; Lugano: B. Baumberger, S. Boccia Soldati, E. Gehrig-Van Essen and S. Ronchetto; Montana: C. Bonvin and C. Burrus; Payerne: S. Blanc, A.V. Ebinger, M.L. Fragnière and J. Jordan; and Wald: R. Gimmi, N. Kourkoulos and U. Schafroth. Administrative staff: N. Bauer (Basel), D. Baehler (Geneva), C. Gabriel (Geneva) and R. Nilly (Basel).

REFERENCES

- Ackermann-Lieblich U, Leuenberger P, Schwartz J, *et al.* Lung function and long term exposure to air pollutants in Switzerland. Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) Team. *Am J Respir Crit Care Med* 1997; 155: 122–129.
- Downs SH, Schindler C, Liu LJ, *et al.* Reduced exposure to PM₁₀ and attenuated age-related decline in lung function. *N Engl J Med* 2007; 357: 2338–2347.
- Abbey DE, Burchette RJ, Knutsen SF, *et al.* Long-term particulate and other air pollutants and lung function in nonsmokers. *Am J Respir Crit Care Med* 1998; 158: 289–298.
- MacNee W. Oxidative stress and lung inflammation in airways disease. *Eur J Pharmacol* 2001; 429: 195–207.
- Cascio WE. Cardiopulmonary health effects of air pollution: is a mechanism emerging? *Am J Respir Crit Care Med* 2005; 172: 1482–1484.
- Fredenburgh LE, Perrella MA, Mitsialis SA. The role of heme oxygenase-1 in pulmonary disease. *Am J Respir Cell Mol Biol* 2007; 36: 158–165.
- Guénégou A, Leynaert B, Bénessiano J, *et al.* Association of lung function decline with the heme oxygenase-1 gene promoter microsatellite polymorphism in a general population sample. Results from the European Community Respiratory Health Survey (ECRHS), France. *J Med Genetics* 2006; 43: e43.
- Siedlinski M, van Diemen CC, Postma DS, *et al.* Heme oxygenase 1 variations and lung function decline in smokers: proof of replication. *J Med Genetics* 2008; 45: 400.
- Ugenskiene R, Sanak M, Sakalauskas R, *et al.* Genetic polymorphisms in chronic obstructive pulmonary disease. *Medicina (Kaunas, Lithuania)* 2005; 41: 17–22.
- Rahman I. Regulation of glutathione in inflammation and chronic lung diseases. *Mutat Res* 2005; 579: 58–80.
- Sundberg K, Johansson AS, Stenberg G, *et al.* Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis* 1998; 19: 433–436.
- Imboden M, Downs SH, Senn O, *et al.* Glutathione S-transferase genotypes modify lung function decline in the general population: SAPALDIA cohort study. *Respir Res* 2007; 8: 2.
- Gilliland FD, Gauderman WJ, Vora H, *et al.* Effects of glutathione-S-transferase M1, T1, and P1 on childhood lung function growth. *Am J Respir Crit Care Med* 2002; 166: 710–716.
- Ackermann-Lieblich U, Kuna-Dibbert B, Probst-Hensch NM, *et al.* Follow-up of the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA 2) 1991–2003: methods and characterisation of participants. *Soz Präventivmed* 2005; 50: 245–263.
- Burney PG, Luczynska C, Chinn S, *et al.* The European Community Respiratory Health Survey. *Eur Respir J* 1994; 7: 954–960.
- Standardisation of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; 152: 1107–1136.
- Martin BW, Ackermann-Lieblich U, Leuenberger P, *et al.* SAPALDIA: methods and participation in the cross-sectional part of the Swiss Study on Air Pollution and Lung Diseases in Adults. *Soz Präventivmed* 1997; 42: 67–84.
- Dreborg S. Skin tests used in type I allergy testing. Position paper of the European Academy of Allergology and Clinical Immunology. *Allergy* 1989; 44: Suppl. 10, 52–59.
- Stephens M, Smith N, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001; 68: 978–989.
- Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003; 73: 1162–1169.
- Liu LJS, Curjuric I, Keidel D, *et al.* Characterization of source-specific air pollution exposure for a large population-based Swiss cohort (SAPALDIA). *Environ Health Perspect* 2007; 115: 1638–1645.
- SAEFL. Modelling of PM₁₀ and PM_{2.5} ambient concentrations in Switzerland 2000 and 2010. Environmental Documentation No 169 Air. Berne, Swiss Agency for the Environment, Forests, and Landscape, 2003.
- Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26: 948–968.
- Kampa M, Castanas E. Human health effects of air pollution. *Environ Pollut* 2008; 151: 362–367.
- Lee YL, Lin YC, Lee YC, *et al.* Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. *Clin Exp Allergy* 2004; 34: 1707–1713.
- Islam T, Berhane K, McConnell R, *et al.* Glutathione-S-Transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. *Thorax* 2008; 64: 197–202.
- Romieu I, Ramirez-Aguilar M, Sienna-Monge JJ, *et al.* GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. *Eur Respir J* 2006; 28: 953–959.
- Melen E, Nyberg F, Lindgren CM, *et al.* Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. *Environ Health Perspect* 2008; 116: 1077–1084.

- 29 Gilliland FD, Li Y-F, Saxon A, *et al.* Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study. *Lancet* 2004; 363: 119–125.
- 30 Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet* 1999; 65: 220–228.
- 31 Roewer L, Croucher PJP, Willuweit S, *et al.* Signature of recent historical events in the European Y-chromosomal STR haplotype distribution. *Hum Genet* 2005; 116: 279–291.
- 32 Kramer CB, Cullen AC, Faustman EM. Policy implications of genetic information on regulation under the Clean Air Act: the case of particulate matter and asthmatics. *Environ Health Perspec* 2006; 114: 313–319.