ERJ Express. Published on September 9, 2009 as doi: 10.1183/09031936.000443-2009

Title:

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

Short title:

HMOX1, *GST*, reduced PM10 and FEF_{25-75}-decline

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Word count abstract: **200**
Word count (body of text without tables & references): **3000**

Keywords:

FEF_{25-75}
General population sample
Glutathione S-transferase
Heme oxygenase-1
Lung function decline
PM10 exposure

Abstract

Study question

Reduced particulate matter (PM10) exposure attenuated the age-related lung function decline in our cohort particularly in the small airways (FEF_{25-75}). We hypothesized that polymorphisms in glutathione S-transferase (*GST*) and heme oxygenase-1 (*HMOX1*) genes, important for oxidative-stress defense, modify these beneficial effects.

Methods

A population-based sample of 4365 adults was followed up 11 years including questionnaire, spirometry, and DNA blood sampling. PM10 exposure was estimated by dispersion modeling and temporal interpolation. Main effects on annual decline in 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.

Results

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

Conclusion

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 defense.

Background

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 defense importantly determine susceptibility to external noxious exposures. Polymorphisms of the *heme 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 catalyzes heme degradation, producing bilirubin, CO and ferritin which exert important anti-oxidative 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 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, gluthatione (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 isoleucin (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 PM10 was associated with a significant attenuation of the age-related decline in lung function over 11 years of follow-up[2]. Attenuations were strongest for the mid-expiratory flow (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 years from eight areas of Switzerland. 9651 persons participated in the first assessment in 1991, and at the follow-up examination in 2002, 8047 were re-assessed. Written consent was obtained from all study participants. Approval of the study was given by the Swiss Academy of Medical Sciences and the regional ethics committees. The present study sample consists of 4365 participants with complete data regarding spirometry, residential history, smoking history, PM10 exposure and available genotyping information (figure 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 persons who at the time of follow-up had smoked less than 20 packs of cigarettes or 360g 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 and ex-smokers, 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). FVC, FEV_1, and forced expiratory flow between 25 and 75% of the FVC (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 3mm 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 supplemental document ‘genotyping procedures’.

Shortly, 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 single nucleotide polymorphisms (SNPs) of the *HMOX1* gene, rs2071746 [T/A], rs5995098 [G/C] and rs735266 [T/A] were genotyped using real time PCR (TaqMan®). *HMOX1* haplotypes were inferred using PHASE software v2.1. Haplotypes with prevalences below 5% were not analyzed.

HMOX1 (GT)n promoter repeats were determined using PCR and fragment size analysis with GeneMapper® software v3.5 (Applied Biosystems, Rotkreuz, Switzerland). 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 was described in detail previously[2, 19]. Each participant was assigned annual average PM10 concentrations for 1991 and 2002 based on his residence address using a Gaussian dispersion model (PolluMap, version 2.0) 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 years of follow-up. PM10 exposure in Switzerland declined throughout the study period, therefore median change in PM10 exposure was negative.

Statistical analysis

A detailed characterization of the study population can be found in online TableO1. To address the influence of self-selected participation, our study sample was compared to all participants with complete data at the baseline examination, but not at follow-up.

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, pack-years up to the baseline and between surveys as cumulative smoking exposure, parental smoking, workplace-exposure to dust and fumes at each survey, 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 $10\mu g/m^3$ decline in PM10 over the average follow-up period of 10.92 years. To control for the influence of FVC on the measurement of FEF_{25-75}[20], we repeated the analysis 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 men and women.

In a sensitivity analysis, we stratified our study sample 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 10000 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, Texas, 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 years, and 29.1% of participants were smokers with a median of 18.4 pack-years smoked. Mean values for FVC at the baseline examination were 4.5 L, for FEV_1 3.6 L and for FEF_{25-75} 3.4 L/s. A more detailed characterisation can be found in online TableO1. 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 FEF_{25-75} during follow-up in the whole study sample are presented in table 2, sex-specific results are shown in online TableO2.

***HMOX1* polymorphisms**

The homozygous mutant SNP-alleles of all three *HMOX1* SNPs and related haplotypes showed mostly favourable effects, attenuating the natural decline in FEF_{25-75} in the whole study sample by up to 5.9 ml/s per year (95%-confidence interval (95%-CI): –0.5 to 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 FEF_{25-75} decline was found for having at least one long (GT)n promoter repeat allele.

***GST* polymorphisms**

GSTP1 was associated with annual FEF_{25-75} 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 4.4mL/s (table 2).

Table 1 Characteristics of study sample and excluded participants

Variable	Baseline participants not included in the current study (n=4685*)	Current study sample (n=4365)	P values Included vs. Not-included
Female sex (%)	50.2	52.9	**0.010**
Age in 1991 (years) *mean/sd*	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-years in smokers 1991 *median/1.quartile/3.quartile*	17.7 / 7.3 / 31.7	18.4 / 8.0 / 31.8	0.470
Pack-years in smokers 2002 *median/1.quartile/3.quartile*	NA /	21.6 / 8.6 / 35.9	
High educational level in 1991 (%)	15.8	17.3	**0.058**
FVC in 1991 (L) *mean/sd*	4.4 1.0	4.5 1.0	**<0.001**
Change in FVC up to 2002 (L) *mean/sd*		-0.3 0.4	
FEV_1 in 1991 (L) *mean/sd***Change in FEV_1 up to 2002 (L)** *mean/sd*	3.5 0.9	3.6 0.8 -0.4 0.3	**0.004**
FEV_1/FVC RATIO >0.7 in 1991 (%)	86.9	89.8	**<0.001**
FEF_{25-75} in 1991 (L/s) *mean/sd*	3.4 1.3	3.4 1.2	0.952
Change in FEF-25-75 up to 2002 (L/s) *mean/sd*		-0.8 0.7	
Change in PM10 exposure during follow-up ($\mu g/m^3$) *median/1.quartile/3.quartile*	NA /	-5.3 / -7.3 / -4.2	

* sample size may decrease up to n=4517 due to missing values

Table 2 Main effects of *HMOX1* and *GST* polymorphisms on change in FEF_{25-75}

Gene-Polymorphism			Gene main effect on decline in FEF_{25-75}/year (in mL/s per year)[1]	95% confidence interval	P-value
***Heme Oxygenase-1* Single Nucleotide Polymorphisms**					
	Alleles	***N***			
***HMOX1* rs2071746**	***AA***	***1401***	ref		
	AT	***2089***	0.8	-3.3 to 4.9	0.053[c]
	TT	***763***	5.1	-0.2 to 10.5	
***HMOX1* rs735266**	***AA***	***1669***	ref		
	AT	***2022***	1.2	-2.7 to 5.1	0.128[a]
	TT	***595***	4.7	-1.0 to 10.4	
***HMOX1* rs5995098**	***CC***	***2021***	ref		
	CG	***1876***	-1.6	-5.4 to 2.2	**0.047[c]**
	GG	***427***	5.3	-1.0 to 11.7	
***Heme Oxygenase-1* Haplotypes[2]**					
	No of alleles	***N***			
Haplotype AAC	***0***	***798***	ref		
	1	***2178***	-3.3	-8.2 to 1.6	0.09[a]
	2	***1389***	-4.7	-10.0 to 0.5	
Haplotype ATC	***0***	***3925***	ref		
	1	***431***	2.0	-4.0 to 8.1	0.476[a]
	2	***9***	5.6	-33.9 to 45.1	
Haplotype TTC	***0***	***3873***	ref		
	1	***478***	4.3	-1.5 to 10.0	0.156[b]
	2	***14***	-2.1	-33.8 to 29.6	
Haplotype TTG	***0***	***2057***	ref		
	1	***1895***	-1.7	-5.5 to 2.1	**0.031[c]**
	2	***413***	5.9	-0.5 to 12.3	
***Heme Oxygenase-1* Promoter Repeat Polymorphism**					
	No of alleles	***N***			
***HMOX1* (GT)n long allele[3]**	***none***	***3919***	ref		
	1 or 2	***446***	-1.1	-7.0 to 4.9	0.728
***Glutathione S-Transferase* Polymorphisms**					
	Complete deletion/ allele	***N***			
***GSTM1* deletion[4]**	***no***	***2032***	ref		
	yes	***2319***	-1.1	-4.7 to 2.5	0.558
***GSTT1* deletion[4]**	***no***	***3554***	ref		

	yes	***797***	-1.1	-5.8	to	3.6	0.644
***GSTP1 Val/Ile* Polymorphism**	***Ile / Ile***	***2061***	ref				
	Ile/Val	***1892***	-3.6	-7.3	to	0.2	**0.044**[b]
	Val/Val	***401***	-4.4	-10.9	to	2.1	

a under an additive genetic model
b under a dominant genetic model
c under a recessive genetic model

[1] Negative values represent accelerations of the natural decline, positive values attenuations, compared to the reference group. Estimates from a multivariable linear regression of annual change in FEF_{25-75} on genetic variants adjusted for: 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 BMI, change in BMI and the interaction between the two, level of education and its change, nationality, season of assessment and clustering within area.

[2] Determined from rs2071746, rs735266 and rs5995098 SNPs (in this sequence) using PHASE software v2.1

[3] HMOX-1 (GT)n long allele is defined as having at least 33 GT repeats

[4] Deletions are homozygous gene deletions (null vs. Non-null).

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 FEF_{25-75} decline are presented in table 3. Sex-specific results are presented in online TableO3 and TableO4. Analyses equivalent to the ones for FEF_{25-75} were done for change in forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC), but only weak interaction signals were found (TableO5).

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 10µg/m^3 during follow-up was significantly associated with an attenuation of the annual decline in FEF_{25-75} by 15.3 ml/s (95%-CI 7.8 to 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 FEF_{25-75} decline by 26.5ml/s (95%-CI 11.7 to 41.2), as opposed to only 11.7ml/s (95%-CI 4.3 to 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 FEF_{25-75} by 27.3ml/s (95%-CI 11.4 to 43.2). Those with one or no Val allele presented attenuations of 9.8ml/s (95%-CI 0.7 to 19.0) and 13.5ml/s (95%-CI 5.2 to 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 FEF_{25-75} adjusted for FVC to calculate change in FEF_{25-75} did not yield different results. Apart from small changes in effect size, the patterns of interaction persisted (Table 3, right hand column).

Table 3 Effect of a decrease in PM10 exposure on change in FEF_{25-75}, by *HMOX* and *GST* genotypes

Gene Polymorphism			Effect of a 10µg/m³ decrease in PM10 over 10.92 years (mean follow-up) on annual change in FEF_{25-75} (mL/s per year) [1]		annual change in FEF_{25-75} (mL/s per year) adjusted for FVC [1,2]	
			Estimate	95%-confidence interval	Estimate	95%-confidence interval
***Heme Oxygenase-1* Single Nucleotide Polymorphisms**						
	allele	*N*				
HMOX 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
HMOX 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
HMOX 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
***Heme Oxygenase-1* Haplotypes[3]**						
	No of alleles	***N***				
Haplotype AAC	***0***	*798*	10.6	-1.5 to 22.8	12.5	-0.4 to 25.4
	1	*2178*	14.2	5.5 to 22.9	15.7	6.5 to 25.0
	2	*1389*	13.1	3.9 to 22.3	13.5	3.6 to 23.3
Haplotype ATC	***0***	*3925*	**15.3** [a]	**7.8 to 22.7**	**16.5** [b]	**8.6 to 24.4**
	1	*431*	-4.3	-18.9 to 10.4	-3.3	-18.9 to 12.3
	2	*9*	31.9	-62.3 to 126.1	8.8	-91.3 to 109.0
Haplotype TTC	***0***	*3873*	13.0	5.6 to 20.5	14.0	6.1 to 21.9
	1	*478*	11.1	-3.9 to 26.0	14.0	-2.0 to 29.9
	2	*14*	36.5	-9.8 to 82.8	36.9	-12.4 to 86.1
Haplotype TTG	***0***	*2057*	11.2	3.0 to 19.5	11.9	3.1 to 20.6
	1	*1895*	17.2	7.9 to 26.5	18.9	9.0 to 28.7
	2	*413*	10.7	-5.0 to 26.3	12.6	-4.0 to 29.2
***Heme Oxygenase-1* Promoter Repeat Polymorphism**						

	No of alleles	N				
HMOX $(GT)_n$	***none***	*3919*	11.7	4.3 to 19.2	12.8	4.9 to 20.7
long allele[4]	***1 or 2***	*446*	**26.5 [c]**	**11.7 to 41.2**	**27.5 [d]**	**11.8 to 43.2**
***Glutathione S-Transferase* Polymorphisms**						
	complete deletion / allele	***N***				
GSTM1 deletion[5]	***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
GSTT1 deletion[5]	***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
GSTP1 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 [e]**	**11.4 to 43.2**	**26.7 [f]**	**9.8 to 43.7**

[a] $p_{interaction}=0.009$, and [b] $p_{interaction}=0.011$ under dominant genetic model
[c] $p_{interaction}=0.044$, and [d] $p_{interaction}=0.06$
[e] $p_{interaction}=0.052$, and [f] $p_{interaction}=0.107$ under recessive genetic model

[1] Positive values represent attenuations of annual natural decline in FEF_{25-75}, compared to 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 smokers at both surveys, pack-years up to baseline and between surveys, parental smoking, workplace-exposure to dust and fumes at each survey, baseline BMI, change in BMI and the interaction between the two, level of education and its change, nationality, season of assessment and clustering within area.

[2] Adjustment for FVC was done by regressing FEF_{25-75} on FVC and using residuals for calculation of the change.

[3] Determined from rs2071746, rs735266 and rs5995098 SNPs (in this sequence) using PHASE software v2.1

[4] HMOX-1 (GT)n long allele is defined as having at least 33 GT repeats[5]
Deletion means homozygous gene deletion (null vs. Non-null)

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 men (figure 3), while that with long *HMOX1* $(GT)_n$ promoter repeat alleles was only seen in women. Genotype specific PM10-effects are presented in online TableO3 and TableO4.

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 *HOMX1* haplotype ATC were p=0.009 (dominant genetic model), p=0.041 for a long *HMOX1* (GT)n promoter repeat and 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* valin 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 emphasized effect on FEF_{25-75} decline may be due to preferential deposition of the fine, more health-relevant portion of PM10 (PM2.5 and smaller) in smaller airways[21], and is not unexpected as PM10 reduction affected FEF_{25-75} decline most strongly in our previous work[2].

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[22, 23]. In contrast, *GSTP1* valine alleles were associated with respiratory symptoms in children highly exposed to ozone and with allergic sensitization in adults exposed to traffic air pollution[24, 25]. Experimental studies in ragweed-sensitized adults showed stronger inflammatory reactions upon allergen exposure for *GSTM1*-null or *GSTP1* 105Ile genotypes only in co-presence of diesel exhaust or environmental tobacco smoke[26]. 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, 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 not-included cohort subjects. 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 (HWE) 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[27], but since genetic homogeneity of Caucasian Western-Central European populations has been described[28], 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 characterized *HMOX1* genetic variants.

Finally, it was beyond the scope of this current study to investigate how modifiable life style 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[29].

Acknowledgements

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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, D Gashi, Basel: R Armbruster, T Damm, U Egermann, M Gut, L Maier, A Vögelin, L Walter, Davos: D Jud, N Lutz, Geneva: M Ares, M Bennour, B Galobardes, E Namer, Lugano: B Baumberger, S Boccia Soldati, E Gehrig-Van Essen, S Ronchetto, Montana: C Bonvin, C Burrus, Payerne: S Blanc, AV Ebinger, ML Fragnière, J Jordan, Wald: R Gimmi, N Kourkoulos, U Schafroth.

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Funding

The Swiss National Science Foundation (grants no 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, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino,

Zurich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino and Zurich, UBS Wealth Foundation Grant BA29s8Q7-DZZ.

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Figure 1 Selection of Study participants

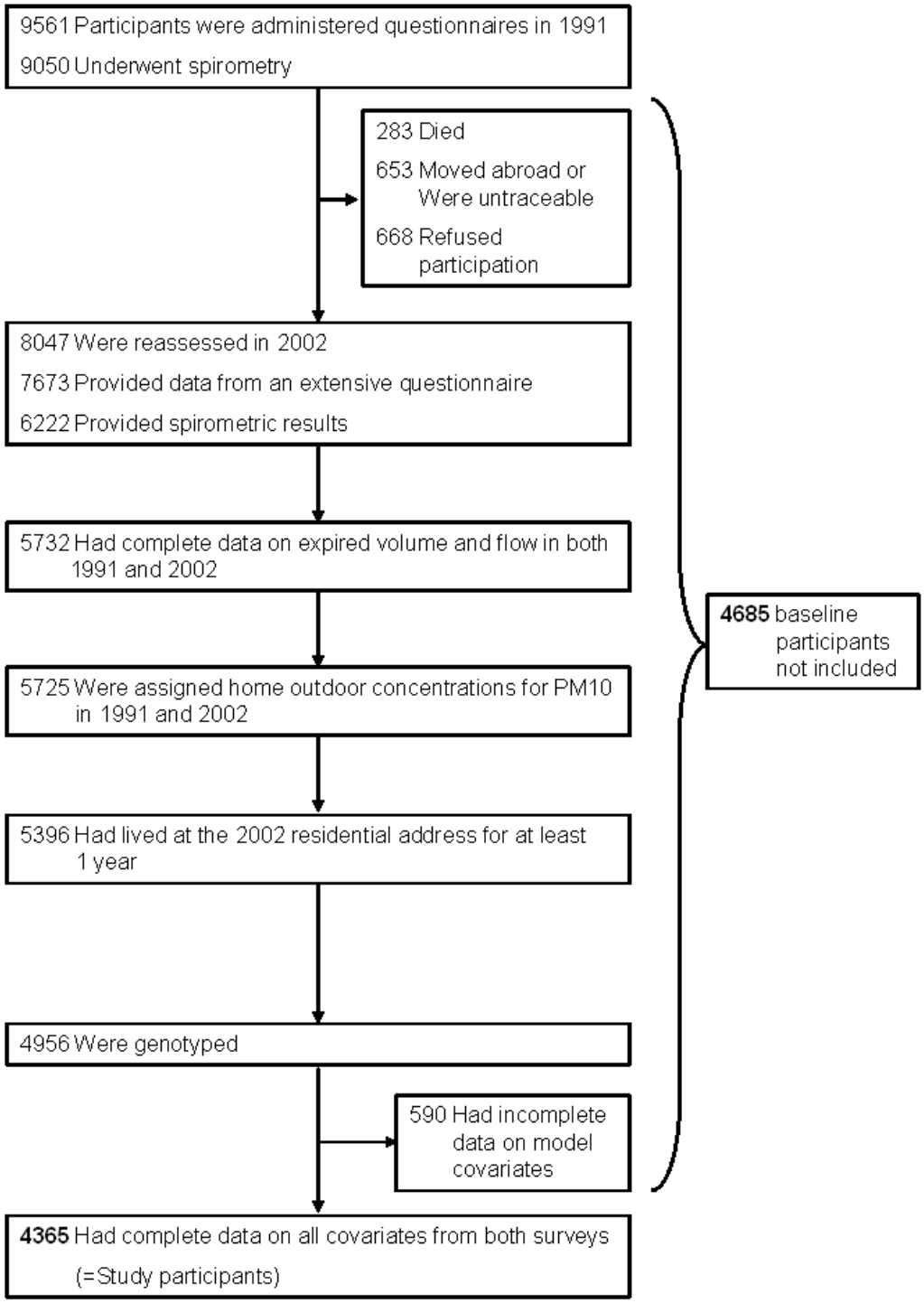


Figure 2 Distribution of $(GT)_n$ repeats of the *HMOX1* promoter polymorphism

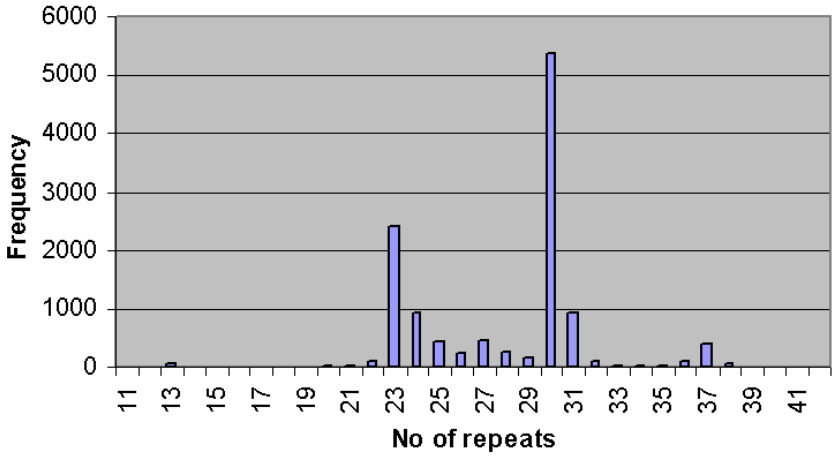
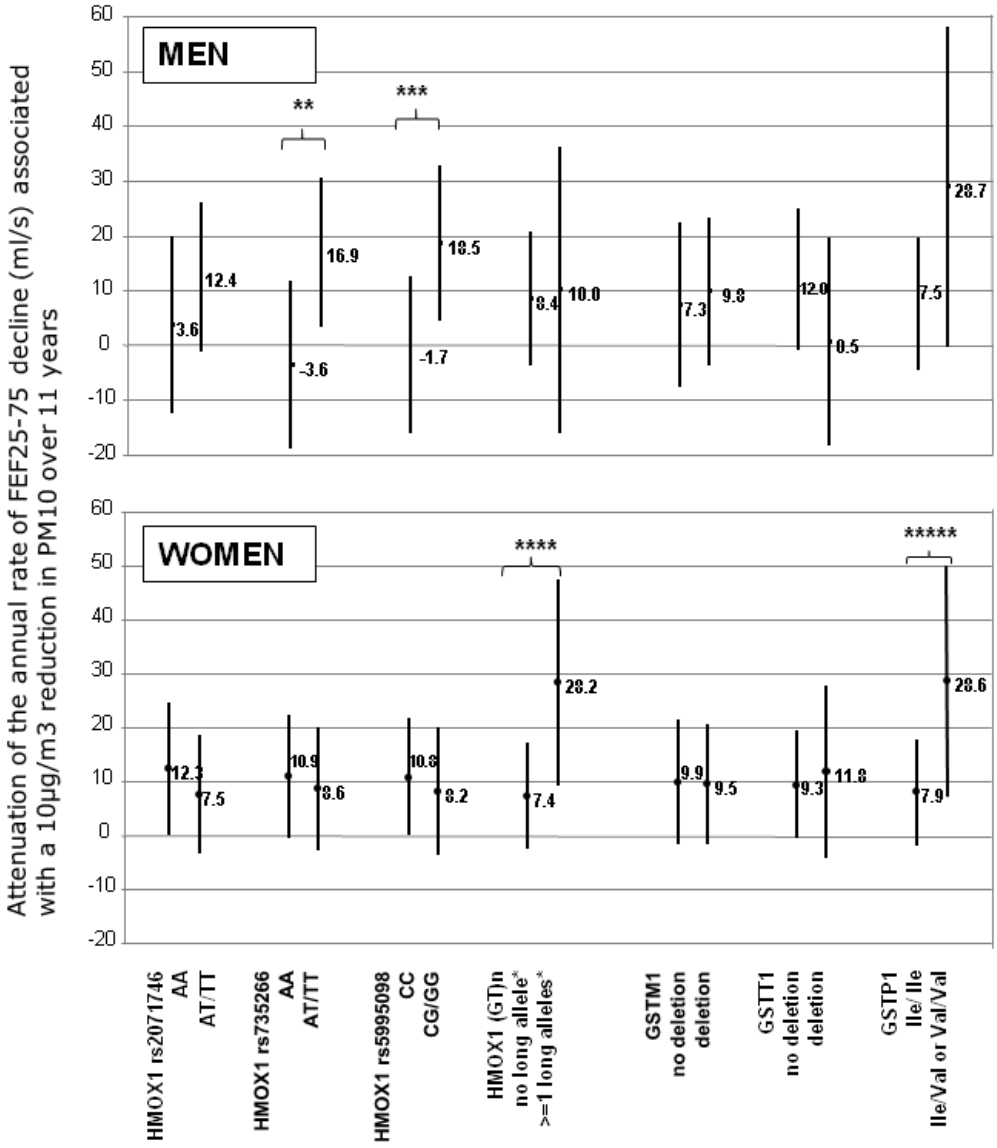


Figure 3 Estimated attenuation of the annual rate of decline in FEF_{25-75} associated with a 10µg/m³ decline in PM10 over 11 years of follow-up, stratified by genotype and sex



Positive values indicate attenuation of decline in FEF_{25-75}

*: Long allele: ≥33 GT promoter repeats; **: $P_{interaction}$=0.008 assuming dominant genetic effects; ***: $P_{interaction}$=0.007 assuming dominant genetic effects; ****: $P_{interaction}$=0.028; *****: $P_{interaction}$=0.052 assuming recessive genetic effects