

ORIGINAL ARTICLE

Effect modification of immunoglobulin E-mediated atopy and rhinitis by glutathione S-transferase genotypes in passive smokers

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Summary

Background Experimental studies suggest that glutathione S-transferase (GST) genotypes modify nasal allergen responses induced by secondhand smoke (SHS) exposure.

Objective We aimed to investigate whether GSTs affected systemic IgE and allergic rhinitis (AR) in SHS-exposed individuals from a population-based cohort.

Methods Analyses comprised 2309 never-smokers from the Swiss study on air pollution and health in adults cohort, reporting SHS status at baseline and 11 years later. Outcomes were defined by total serum IgE ≥ 100 kU/L, specific serum IgE determined by Phadiatop[®] ≥ 0.35 kU/L and self-reported AR. *GSTP1* Ile105Val, *GSTM1* and *GSTT1* gene deletion genotypes were identified at the follow-up survey.

Results After adjustment for relevant covariates, the homozygous *GSTP1* 105-Val genotype was negatively associated with high total IgE and high-specific IgE by Phadiatop[®], notably in subjects persistently exposed to SHS (OR: 0.20, 95% CI 0.05–0.75; $P = 0.02$, for high total IgE and OR: 0.29, 95% CI 0.10–0.89; $P = 0.03$, for high specific IgE by Phadiatop[®]). Carrying at least one copy of the *GSTM1* gene (non-null) showed a similar association for high specific IgE by Phadiatop[®] (OR: 0.41, 95% CI 0.22–0.76; $P = 0.004$). No significant associations were found between GSTs and rhinitis.

Conclusion and Clinical Relevance In this large cohort, homozygosity for *GSTP1* 105-Val or carrying the *GSTM1* non-null genotype decreased the risk of high total IgE or high specific IgE using Phadiatop[®] by nearly half in subjects exposed to SHS, as compared with subjects carrying opposite alleles. These findings underline the value of genetic susceptibility when evaluating the effects of environmental exposure on allergic illness. The potential long-term effects of persistent SHS exposure in genetically vulnerable individuals may be of public health relevance.

Keywords allergy, cohort studies, genetic polymorphisms, glutathione-S-transferase, passive smoking, rhinitis

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Introduction

Involuntary exposure to tobacco smoke has been systematically reported as an important determinant of respiratory morbidity, notably in vulnerable individuals such as asthmatics [1–6]. Many studies in the literature have also focused on the effects of exposure to parental smoking and the development of allergic sensitization and respiratory illness in children [7–12]. In adults, it has been

established that increased specific IgE is a determining factor associated with the onset of asthma [13]. Moreover, increased specific IgE levels were found in the nasal lavages of subjects exposed to tobacco smoke in an experimental model [14]; this suggests that secondhand smoke (SHS) enhances IgE-mediated allergic responses in the upper airways. However, the role of SHS exposure in atopy is less clear. Studies investigating the association between SHS exposure and serum IgE levels as markers of

atopy have been inconclusive [15, 16]. Among factors with the potential to explain some of the inconsistencies found in the literature, differences in individuals' characteristics may be of relevance. A growing body of evidence now indicates that SHS susceptibility might be modified by genetic variation.

Glutathione S-transferases (GST) are a group of enzymes with characteristics that have been reported to neutralize the effects of tobacco smoke and to reduce oxidative stress [17]. Most studies addressing genetically associated respiratory susceptibility have focused on *GSTP1*, the most abundant GST isozyme in the lungs, as well as *GSTM1* and *GSTT1*, both of which are missing in considerable fractions of the population due to gene deletions. Several studies have reported the effect modulation features of GST genotypes on SHS-induced respiratory symptoms and asthma in children. The available evidence consistently points to an increased risk for asthma and respiratory symptoms in children lacking the *GSTM1* gene [18–20] and, more recently, in carriers of the *GSTP1* isoform with a valine at position 105 (*GSTP1* 105-Val) instead of the isoleucine (*GSTP1* 105-Ile) [21]. However, these results contrast with findings in adults, which demonstrate a protective effect of the valine allele in asthma [22–24]. A large body of literature has addressed the adverse effects of involuntary exposure to SHS. However, few studies have investigated the potential interplay between GSTs and SHS with regard to atopy in adults, an association with potential public health relevance. A single study has reported this association in a controlled nasal challenge model that included 19 subjects [25]. The results from this experimental study showed increased specific IgE levels in *GSTP1* 105-Ile and *GSTM1*-null carriers following short local SHS exposure. However, it is unclear whether SHS exposure affects serum IgE levels as systemic markers of atopy. In addition, it is still unknown whether experimental observations remain in a population-based context. In the present study, we aimed to investigate whether *GSTP1*, *GSTM1* and *GSTT1* polymorphisms are associated with susceptibility to IgE-determined atopic responses and allergic rhinitis (AR) in participants from the Swiss study on air pollution and health in adults (SAPALDIA) cohort exposed or not to SHS. In addition, we examined whether these associations changed in relation to the persistence of SHS exposure over time.

Methods

The Swiss study on air pollution and health in adults study

SAPALDIA is a population-based cohort study designed to investigate and monitor the impact of indoor and outdoor environmental airborne pollution on the respiratory and cardiovascular health of the Swiss population. For this

purpose, adults from eight areas of the country were randomly selected from the population registries to represent the climatic, geographical and cultural diversity of Switzerland. A total of 9651 individuals participated in the first survey in 1991 (SAPALDIA 1), and 83% of the original cohort was re-examined during the follow-up survey in 2002 (SAPALDIA 2). Details on the health assessments performed during these occasions have been described elsewhere [26, 27]. Briefly, during a 2-h visit, participants answered a standardized computer-based health interview administered by trained fieldworkers, which covered general health status, respiratory symptoms, allergies, exposure to tobacco smoke and occupational exposure to airborne hazards. At the same occasion, blood samples were collected for, among other tests, total serum IgE and specific IgE measured using the Phadiatop[®] test (Pharmacia Diagnostics, Uppsala, Sweden). DNA genotyping was performed at the follow-up survey.

Selection of subjects and definition and categorization of secondhand smoke exposure

Of the original 8047 subjects participating in both surveys, 2463 never-smokers who had agreed to blood tests and DNA genotyping and had complete data on outcomes and covariates were eligible for the study. Exposure to SHS was categorized in three groups: (1) subjects never exposed to SHS, (2) subjects having reported SHS exposure at the baseline survey but no longer at follow-up and (3) subjects having reported SHS exposure at baseline and follow-up surveys. One hundred and fifty-four subjects who reported exposure to SHS only at the follow-up survey, in whom longitudinal SHS effects could not be assessed, were excluded from analyses.

Out of the 2309 never-smokers who were eligible for analyses, 1630 (70%) claimed no exposure to SHS in both surveys, 453 (20%) reported exposure at the first survey and 226 (10%) reported exposure at both surveys 11 years apart. Among the subjects exposed to SHS, 10% reported weak exposure (i.e. during <3 h/day at baseline and no exposure at follow-up), while 4% reported strong SHS exposure (i.e. being exposed during more than 3 h/day in both surveys). All other subjects (15%) were considered to have medium exposure.

Never-smokers were defined as participants who had never smoked or had smoked fewer than 20 packs of cigarettes or <360 g of tobacco in their lifetime. Exposure to SHS was assessed at each survey by the question 'Have you been regularly exposed to tobacco smoke in the last year?' The assessment of tobacco consumption and SHS exposure using the health questionnaire was validated in the context of the European Community Respiratory Health Survey (ECRHS) [28]. SAPALDIA has a close link to the ECRHS through its common study centre, Basel, and the two studies essentially share the same questionnaire.

Blood sampling, immunoglobulin-E measurements and genotyping

A detailed description of the collection, fractioning and storing of blood aliquots during the SAPALDIA baseline and follow-up surveys has been published previously [26, 27]. Briefly, blood samples from all participants who had given informed consent for genetic and allergy serum marker analysis were drawn, processed and stored at -80°C in each of the study centres. For safety reasons, at the end of each survey, the collection of biological samples was divided and stored in two distinct centres located at the University Hospitals of Zürich and Geneva, connected to a central alarm with permanent technical maintenance.

Serum samples in both surveys were collected for total IgE and Phadiatop[®] tests. Total serum IgE was measured using the CAP FEIA System (Pharmacia Diagnostics). Specific IgE was measured by the *in vitro* Phadiatop[®] technique using fluoroenzyme immunoassays (CAP FEIA system, Pharmacia Diagnostics). This allergy-screening test detects the presence of specific serum IgE antigens against common inhalant allergens, including birch, timothy grass, ragweed, cat, dog, horse, *Dermatophagoides pteronyssinus* and *Aspergillus fumigatus*. The results are based on the Phadiatop[®] percent binding, according to the reference serum value threshold. Details on the Phadiatop[®] technique and its validation in SAPALDIA have been reported previously [13, 29].

EDTA-buffered whole blood for DNA extraction was collected only at the follow-up survey (SAPALDIA 2). Manual extraction of DNA was performed using the Gentra[™] Puregene Kit (GENTRA Systems, Minneapolis, MN, USA). All subjects were genotyped for *GSTM1* (UniGene ID Hs.301961; UniGene 2008a) and *GSTT1* (UniGene Hs.268573; UniGene 2008b) gene deletions and a single nucleotide polymorphism (SNP) in *GSTP1* (UniGene Hs.523836; UniGene 2008c) that results in the amino acid substitution Ile105Val (rs1695). Genotyping of the *GSTP1* Ile105Val SNP and the gene deletion polymorphisms for *GSTM1* and *GSTT1* was performed using the TaqMan[®] methodology (Applied Biosystems, Rotkreuz, Switzerland), as described previously [30]. The genotype distribution of the *GSTP1* Ile105Val polymorphism was found to be in Hardy-Weinberg equilibrium using Arlequin Version 2.000. Genotype frequencies did not differ by Swiss language region or subjects' nationality.

Outcome variables and definitions

Outcome variables for atopy were analysed independently and consisted of (1) high total serum IgE (level ≥ 100 kU/L) and (2) positive Phadiatop[®] (level ≥ 0.35 kU/L). Atopy was considered when the definition criteria were met in at least one examination (baseline or follow-up survey). Moreover, self-reported AR [31] was analysed as a clinical end-point. The presence of AR was based on the informa-

tion obtained from the health questionnaire at both surveys and defined by a positive answer to the question 'Do you have any nasal allergies including hay fever?' IgE and Phadiatop[®] measurements were obtained at baseline and follow-up surveys.

Statistical analyses

Descriptive statistics are presented as mean $\pm$ SD for quantitative variables and percentages for categorical variables. Differences between groups in relevant general characteristics were assessed using the Wilcoxon Rank-Sum Test for continuous variables and Fisher's exact test for categorical variables.

Analyses were performed on a stepwise basis. First, the main effect models were built to assess the effect of SHS exposure and GSTs on the atopy markers IgE and Phadiatop[®] including the following covariates: area of study, age, sex, body mass index, level of education, occupational exposure to vapours/gases/dusts/fumes and exposure to maternal smoking. Second, the effect of SHS exposure over time on atopy outcomes was analysed using multivariable logistic regression models. Associations of *GSTP1*, *GSTM1* and *GSTT1* genotypes, respectively, with the presence of atopy, as defined above, were analysed in three groups of subjects: (A) subjects never exposed to SHS; (B) subjects exposed to SHS in 1991 (baseline survey; SAPALDIA 1), irrespective of SHS exposure at follow-up and (C) subjects exposed to SHS in 1991 and 2002 (baseline and follow-up surveys; SAPALDIA 1 and 2). Potential modification of the effect of GST genotypes on atopy by SHS exposure over time was tested by including interaction terms in the models. Finally, we performed a sensitivity analysis after excluding all subjects who reported asthma confirmed by a doctor at baseline.

In separate analyses, additional models were conceived to assess interactions and to avoid the need for multiple testing, notably when using several categories of GST genotypes and SHS exposure. For this purpose, we *a priori* collapsed the two *GSTP1* categories Ile/Val and Val/Val into one category, thus enabling to reduce the number of interaction analyses to three (one for each GST genotype – *GSTP*, *GSTM* and *GSTT*). In addition, we considered a four-level trend variable for SHS, assigning '0' to 'no SHS' at baseline or follow-up, '1' to 'weak SHS exposure', '2' to 'moderate SHS exposure' and '3' to 'strong SHS exposure, as defined previously. We also considered an overall model with the previously described GST score for the number of GST risk alleles (i.e. *GSTM1* null = 1 risk allele, *GSTT1* null = 1 risk allele, *GSTP1* Ile/Val = 1 risk allele, *GSTP1* Val/Val = 2 risk alleles), the trend variable summarizing the level of SHS exposure and the interaction between these two variables.

A *P*-value ≤ 0.05 was considered for statistical significance. SAS[®] Version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

Results

The details of the selection of the current study population and the comparison between participants and non-participants in the follow-up survey are detailed in the supporting information. Table 1 shows the characteristics at baseline (SAPALDIA 1) of subjects included in the study, stratified by SHS-exposure status. Overall, subjects exposed to SHS were younger, less likely to have achieved a higher education and more likely to have been exposed to maternal smoking during childhood and to report occupational exposure to airborne hazards.

Overall, we found significant negative associations of homozygous *GSTP1* 105-Val with total serum IgE (OR: 0.62; 95%CI: 0.43–0.90; $P=0.01$) and Phadiatop[®] (OR: 0.71; 95%CI: 0.51–0.98; $P=0.04$). No significant

associations were found between *GSTM1* or *GSTT1* and high total IgE or high specific IgE by Phadiatop[®].

Table 2 displays the adjusted associations between GST genotypes and high total IgE, within each SHS exposure category. Significantly lower odds for high total IgE were observed in homozygous *GSTP1* 105-Val carriers. Table 3 shows similar results for associations between GST genotypes and high specific IgE by Phadiatop[®]. Associations were generally stronger in subjects reporting persistent exposure to SHS at both surveys. These effects were not sensitive to the exclusion of subjects with reported doctor-diagnosed asthma (data not shown).

After introducing interaction terms between SHS exposure and GST genotypes in the respective models, SHS exposure was significantly associated with Phadiatop[®] ($P=0.01$), and marginally so with total serum IgE ($P=0.08$).

Table 1. Characteristics of subjects at baseline (1991), stratified by secondhand smoke (SHS) exposure status

	No SHS <i>n</i> = 1630	SHS at baseline only <i>n</i> = 453*	SHS at baseline and follow-up <i>n</i> = 226
Age (years), mean±SD	41.6±11.9	39.1±13.2 [‡]	39.1±11.9 [‡]
Female gender (%)	59.8	54.1 [†]	58.0
BMI (kg/m ²), mean±SD	23.4±3.4	23.8±3.5	24.1±3.9 [‡]
Lower education (%)	11.9	15.2	18.6 [‡]
Maternal smoking (%)	7.2	16.3 [‡]	15.9 [‡]
Doctor-diagnosed asthma (%)	6.5	7.5	6.6
Occupational exposure (%) [¶]	28.0	31.6	41.5 [‡]
Total IgE (kU/L), mean±SD	78.9±161.5	101.9±226.5	98.5±238.8
Total IgE ≥ 100 kU/L (%)	19.8	21.9	19.7
Phadiatop [®] ≥ 0.35 kU/L (%)	30.8	34.2	30.3
Allergic rhinitis and/or hay fever (%)	22.7	22.9	18.9

*Indicates subjects exposed to SHS only at baseline.

[†] $P<0.05$.

[‡] $P<0.01$ compared with the reference group (no SHS exposure).

[¶]Occupational exposure to vapours/dusts/fumes/gases.

BMI, body mass index; SHS, secondhand smoke.

Table 2. Adjusted* associations between high[†] serum total IgE levels and GST polymorphisms in never smokers, according to SHS exposure status

	No SHS			SHS at baseline			SHS at baseline and follow-up		
	<i>n</i> [‡]	OR (95% CI)	<i>P</i> -value	<i>n</i> [‡]	OR (95% CI)	<i>P</i> -value	<i>n</i> [‡]	OR (95% CI)	<i>P</i> -value
<i>GSTP1</i>									
Ile/Ile	743	Reference		322	Reference		100	Reference	
Ile/Val	726	0.97 (0.77; 1.24)	0.83	279	0.76 (0.52; 1.10)	0.15	95	0.52 (0.26; 1.05)	0.07
Val/Val	145	0.69 (0.44; 1.08)	0.11	62	0.48 (0.23; 0.97)	0.04	26	0.20 (0.05; 0.75)	0.02
<i>GSTM1</i>									
Null	841	Reference		342	Reference		110	Reference	
Non-null	774	1.05 (0.84; 1.32)	0.67	320	0.81 (0.56; 1.16)	0.25	111	0.60 (0.30; 1.19)	0.14
<i>GSTT1</i>									
Null	314	Reference		116	Reference		36	Reference	
Non-null	1301	1.13 (0.84; 1.52)	0.41	546	1.28 (0.79; 2.08)	0.32	185	0.60 (0.26; 1.40)	0.24

*Adjusted for age, sex, body mass index, level of education, study area, maternal smoking and occupational exposure to vapours/dusts/fumes/gases.

[†]Total IgE ≥ 100 kU/L

[‡]Few subjects are missing in different categories. Therefore, the numbers shown in the GST categories do not always add up to the numbers of the entire sample.

OR, odds ratio; CI, confidence interval (lower and upper limits); GST, glutathione S-transferase.

Table 3. Adjusted* associations between high[†] serum Phadiatop[®] levels and GST polymorphisms in never smokers, according to SHS exposure status

	No SHS			SHS at baseline			SHS at baseline and follow-up		
	<i>n</i> [‡]	OR (95% CI)	<i>P</i> -value	<i>n</i> [‡]	OR (95% CI)	<i>P</i> -value	<i>n</i> [‡]	OR (95% CI)	<i>P</i> -value
<i>GSTP1</i>									
Ile/Ile	748	Reference		328	Reference		102	Reference	
Ile/Val	735	1.27 (1.02; 1.59)	0.03	286	0.93 (0.67; 1.30)	0.66	98	0.96 (0.53; 1.75)	0.90
Val/Val	146	0.85 (0.57; 1.25)	0.40	66	0.52 (0.29; 0.94)	0.03	26	0.29 (0.10; 0.89)	0.03
<i>GSTM1</i>									
Null	854	Reference		353	Reference		114	Reference	
Non-null	776	1.04 (0.84; 1.28)	0.70	326	0.78 (0.56; 1.07)	0.13	112	0.41 (0.22; 0.76)	0.004
<i>GSTT1</i>									
Null	319	Reference		118	Reference		36	Reference	
Non-null	1311	1.34 (1.02; 1.76)	0.03	561	1.02 (0.67; 1.56)	0.91	190	0.75 (0.35; 1.61)	0.45

*Adjusted for age, sex, body mass index, level of education, study area, maternal smoking and occupational exposure to vapours/dusts/fumes/gases.

[†]Phadiatop[®] ≥ 0.35 kU/L.

[‡]Few subjects are missing in different categories. Therefore, the numbers shown in the GST categories do not always add up to the numbers of the entire sample.

OR, odds ratio; CI, confidence interval (lower and upper limits); GST, glutathione S-transferase.

As an additional analysis to test all three genotypes simultaneously combined, we considered for each subject the number of risk alleles (i.e. *GSTM1* null, *GSTT1* null, *GSTP1* Val accounting for one risk allele, with *GSTP1* Val/Val accounting for two risk alleles) as an outcome variable and a four-level SHS trend variable (i.e. with categories no/weak/moderate/strong exposure) as an exposure variable. This approach also allowed to reduce the number of parallel comparisons. The analysis showed a significant interaction between these two variables for high specific IgE by Phadiatop[®] ($P = 0.03$).

Among subjects reporting AR, nearly 70% had a positive skin prick test at baseline, 17% reported physician-diagnosed asthma, 34% had positive total serum IgE and 78% had positive specific IgE tested by Phadiatop[®]. Consistent IgE results were observed between surveys. For total serum IgE, 75% and 13% of subjects were, respectively, positive or negative at both surveys; for Phadiatop[®], 63% and 27% of subjects were respectively positive or negative at both surveys. No significant associations were found between AR and GST genotypes in the three categories of SHS exposure, but the direction of genotype effects is consistent with the findings observed for total IgE and specific IgE by Phadiatop[®] (see supporting information).

Discussion

In the present study, we aimed to investigate the epidemiologic relevance of GST polymorphisms on individuals' susceptibility to IgE-mediated atopy in relation to SHS exposure. We show that, among subjects persistently exposed to SHS, homozygous *GSTP1* 105-Val carriers and subjects carrying at least one copy of the *GSTM1* gene have significantly reduced odds for high total

IgE or high specific IgE by Phadiatop[®] compared with subjects featuring homozygous *GSTP1* 105-Ile or *GSTM1* null genotypes. In other words, subjects carrying the homozygous *GSTP1* 105-Val and the non-null *GSTM1* genotypes were at a lower risk of presenting high total IgE or high specific IgE by Phadiatop[®] when exposed to SHS.

Our findings are in line with a previous experimental study showing decreased nasal specific IgE and histamine levels after SHS challenge in heterozygous *GSTP1* Ile105-Val carriers compared with homozygous *GSTP1* 105-Ile carriers [25]. In our study, we were able to expand these findings by reporting similar results at a population level and by showing that homozygous *GSTP1* 105-Val carriers exposed to SHS had a lower atopy risk than homozygous *GSTP1* 105-Ile carriers.

The detrimental effects of involuntary exposure to SHS have been largely documented, and evidence indicates that these effects are enhanced in susceptible individuals [5, 6, 14, 32–35]. Moreover, controlled experiments confirm that challenge with tobacco smoke triggers allergic and inflammatory responses in allergen-sensitive individuals [36]. The toxicity of a number of substances contained in tobacco smoke is well recognized as the primary insult to the respiratory mucosa, followed by the inflammatory cascade leading to the onset of symptoms. Evidence derived from observations in allergen-sensitive subjects demonstrates that SHS exposure exacerbates allergic responses and the release of inflammatory cells, thus indicating that atopy is part of the inflammatory pathway underlying SHS-induced oxidative stress. GST genotypes are known for their role in catalysing enzyme activity and detoxifying a number of endogenous and exogenous substances, such as the contents of inhaled tobacco smoke. GSTs therefore display an important

antioxidant capacity and play a key role in neutralizing the effects of SHS exposure. However, there is evidence suggesting that this protective effect is restricted to specific genetic variants of GSTs. There are differences in the frequency of GST alleles reported, and the homozygous *GSTP1* 105-Val genotype is consistently the least frequently present in population studies [37]. We previously observed that the distribution of GST genotypes in our population-based cohort [30] is similar to the one reported in other European samples [38, 39]. Among subjects included in this study, the proportions of homozygous *GSTP1* 105-Val and 105-Ile carriers were 9% and 57%, respectively.

The strengths of this study are the systematic and longitudinal follow-up of a large population-based cohort that is representative of the countrywide geographic, environmental and cultural diversity. However, the study also has several limitations. First, the exclusion of nearly 28% of never-smokers lacking complete data on all outcomes and covariates might have been a source of bias. As such, a higher proportion of women among subjects were found to be excluded from the study; however, exclusion of subjects reflected on a more equilibrated distribution of gender among subjects eligible for analyses (data not shown). Second, the genetic analyses were based on subjects participating at baseline and follow-up surveys. The GST genotype distribution observed in our European population was consistent with that observed in ethnically comparable studies. It is thus improbable that non-participation played a role in a potential biased selection of polymorphisms among participants in the follow-up survey, as genotype is unlikely to influence the decision to participate in a cohort. Third, we were unable to expand the analyses to examine the effect of SHS on new-onset atopic sensitization. Few never-smokers in our cohort were negative for both IgE markers of atopy at baseline, and became positive at follow-up ($n = 25$). Finally, we did not take into account the potential effect of regular specific medication intake on outcomes. Although 34.5% of our subjects with asthma reported regular use of specific medication, this group represented a small percentage of the studied group (6.7%), corresponding to 2.3% of the overall subjects included in the study.

In conclusion, our study indicates a decreased risk for high total IgE or high specific IgE by Phadiatop[®] in individuals homozygous for the *GSTP1* 105-Val allele or non-deficient for the *GSTM1* gene. Among *GSTP1* 105-Val/Val genotype carriers, the risk is lowered by nearly three quarters in subjects reporting persistent exposure to SHS. However, this potentially protective effect concerns a small number of persons in the population carrying this genotype, estimated as approximately 10% of the subjects examined in this study. Conversely, we might anticipate increased risks of high total IgE or high

specific IgE by Phadiatop[®] in SHS-exposed individuals carrying hetero- or homozygous *GSTP1* 105-Ile genotypes, a group with a greater prevalence in the general population (~90%). These results underscore the consideration of genetic susceptibility when evaluating the effects of environmental exposure on respiratory and allergic illness. The potential long-term effects of persistent SHS exposure in genetically vulnerable individuals may be of public health relevance and remain to be determined.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Selection of subjects for analyses.

Table S1. Characteristics at baseline of never-smokers included in analyses and never-smokers not participating in the follow-up survey.

Table S2. Adjusted* associations between allergic rhinitis and GST polymorphisms in never smokers, according to SHS exposure status.

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Online supplement

Gerbase M W et al. *Effect modification of IgE-mediated atopy and rhinitis by GST genotypes in passive smokers*

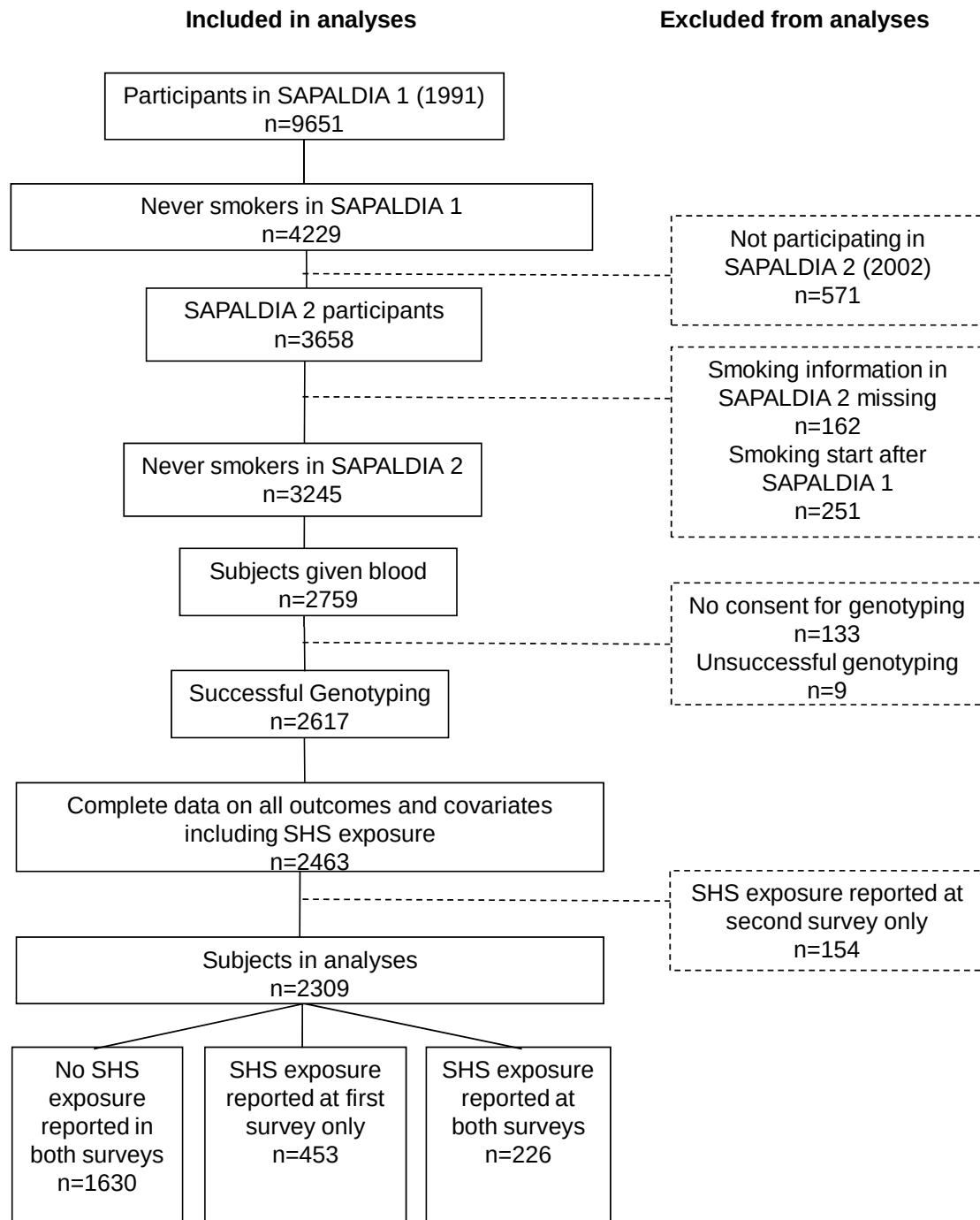


Figure 1. Selection of subjects for analyses

Table 1. Characteristics at baseline of never-smokers included in analyses and never-smokers not participating in the follow-up survey

	Participants in analyses n=2309	Non participants in follow-up survey n=571
Age (years), mean±SD	40.9±12.2	38.9±12.7 [†]
Female sex, %	58.5	58.0
BMI (kg/m ²), mean±SD	23.6±3.5	24.1±4.1 [†]
Lower education, %	13.2	28.8 [†]
Maternal smoking, %	9.8	12.9*
Doctor diagnosed asthma, %	6.7	7.6
SHS exposure, %	29.4	34.7*
Occupational exposure dust/gas/fumes, %	30.0	35.8 [†]
Total serum IgE kU/L, mean±SD	85.3±184.5	92.1±181.2 [†]
Total serum IgE positive, %	20.2	24.1
Phadiatop [®] positive, %	31.4	31.7
Skin Prick Test positive, %	25.2	27.6
Allergic rhinitis and/or hay fever, %	22.3	18.3*

BMI: body mass index ; SHS: secondhand smoke; *p<0.05; [†]p<0.01

Table 1 compares the characteristics at baseline of subjects included in the study analyses and subjects not participating in the follow-up survey. Participants in follow-up were older, had a lower BMI, had achieved higher education, were less exposed to maternal smoking, to SHS and to occupational hazards, had lower total serum IgE levels and were more likely to report allergic rhinitis.

Table 2. Adjusted* associations between allergic rhinitis and GST polymorphisms in never smokers, according to SHS exposure status.

		No SHS			SHS at baseline			SHS at baseline and follow-up		
		n**	OR (95% CI)	p-value	n**	OR (95% CI)	p-value	n**	OR (95% CI)	p-value
<i>GSTP1</i>	Ile/Ile	779	reference		350	reference		111	reference	
	Ile/Val	760	0.94 (0.75;1.18)	0.60	295	1.08 (0.76;1.54)	0.67	101	0.84 (0.45;1.56)	0.58
	Val/Val	153	0.75 (0.49;1.13)	0.16	68	0.65 (0.34;1.25)	0.19	27	0.35 (0.10;1.17)	0.09
<i>GSTM1</i>	Null	888	reference		372	reference		123	reference	
	Non-null	804	0.87 (0.70;1.08)	0.22	341	0.88 (0.62;1.24)	0.45	116	0.71 (0.38;1.31)	0.27
<i>GSTT1</i>	Null	324	reference		125	reference		37	reference	
	Non-null	1368	1.06 (0.80;1.41)	0.67	588	1.21 (0.77;1.91)	0.41	202	0.71 (0.32;1.60)	0.41

*Adjusted for age, sex, body mass index, level of education, study area, maternal smoking and occupational exposure to vapors/dusts/fumes/gases;

**Few subjects are missing in different categories. Therefore, the numbers shown in the GST categories don't always add up to the numbers of the entire sample.