Glutathione S-transferase genotype increases risk of progression from bronchial hyperresponsiveness to asthma in adults

M Imboden, T Rochat, M Brutsche, C Schindler, S H Downs, M W Gerbase, W Berger, N M Probst-Hensch, the SAPALDIA Team

ABSTRACT

Background: Bronchial hyperresponsiveness (BHR) and variation in glutathione S-transferase (GST) genes have been associated with asthma risk. The relationship of these two risk factors with adult onset asthma in the general population was investigated.

Methods: GSTP1 Ile105Val single nucleotide polymorphism and GSTM1 and GSTT1 gene deletion polymorphisms were genotyped in the population-representative SAPALDIA cohort. BHR was assessed at baseline by methacholine challenge and defined as a fall of ≥20% in forced expiratory volume in 1 s. Independent effects of GST polymorphisms and BHR on new onset of asthma after 11 years of follow-up were estimated by multiple logistic regression analysis, adjusting for relevant baseline measures. Effect modification was assessed by including interaction terms in the model.

Results: Among 4426 asthma-free participants at baseline, 14% had BHR. At follow-up, 3.3% reported new onset of physician-diagnosed asthma. BHR (p = 0.001) and GSTP1 Ile105Val genotype (p = 0.005) were independently associated with incident asthma, but no association was seen for GSTT1 and GSTM1 gene deletion polymorphisms. Among subjects free of respiratory symptoms at baseline, the effect of BHR on the risk of physician-diagnosed asthma at follow-up was restricted to GSTP1 105 Ile/Ile carriers (OR 4.57, 95% CI 2.43 to 8.57 vs 1.40, 95% CI 0.58 to 3.39; p for interaction = 0.023).

Conclusions: If confirmed by independent studies, our results suggest that GSTP1 Ile105Val genotype strongly determines the progression of BHR to physician-diagnosed asthma in the general population.

METHODS

Study population

The SAPALDIA cohort, a prospective multicentre study representative of the adult Swiss general population, investigates environmental and genetic factors determining lung health. Baseline and follow-up examinations 11 years later have previously been described in detail. Briefly, 9561 subjects predominantly of European Caucasian ethnicity aged 18–60 years were examined at baseline in 1991. Of these, 8047 (84%) agreed to participate fully or partly at the follow-up survey in 2002. Information on health and life style was collected by computer-assisted personal interview at both time points and lung function was evaluated using the same spirometer devices (Sensormedics model 2200, Yorba Linda, USA) at both time points.
common airborne allergens was assessed by skin prick test at baseline. Circulating serum levels of total IgE and Phadiatop test for detection of allergen-specific IgE were measured at baseline using the CAP FEIA system (Pharmacia Diagnostics, Uppsala, Sweden). Blood for DNA extraction and informed consent to genetic testing was collected at follow-up.

**Methacholine challenge**

BHR was assessed by methacholine challenge (Provocholine, Roche, Nutley, New Jersey, USA) in 7126 participants at baseline. Increasing concentrations of methacholine (0.59, 1.56, 6.25 and 25.0 mg/ml solutions in a phosphate buffer without phenol) were administered through an aerosol dosimeter (Mefar MB3, Bovezzo, Italy).13 The presence of BHR was defined as a fall of ≥20% in forced expiratory volume in 1 s (FEV1) up to a cumulative dose of 2 mg (8.57 μmol). The methacholine responsiveness was determined for each subject by calculating individual dose-response slopes similar to the method suggested by O’Connor et al.22–25 The slope was then defined as the ratio between the percentage decline in FEV1 and the total cumulative dose of methacholine.

**Definition of asthma incidence, respiratory symptoms and selected covariates**

Incident asthma cases were defined as new asthma reports when participants without asthma at baseline gave a positive answer at follow-up to the following two questions: “Have you ever had asthma?” and “Was this confirmed by a doctor?”.

Current asthma symptoms were defined by a self-report of physician-diagnosed asthma and a positive answer to at least one of the following questions: “Are you currently taking any medication for asthma?” and “Have you had an attack of asthma in the last 12 months?”.

Atopy was defined as a positive skin prick test reaction to at least one common allergen tested.24 Occupational exposure to inhalant irritants was assessed by asking: “Have you ever worked in a job which exposed you to vapours, gas, dust or fumes?”. Smokers were participants who had smoked ≥20 packs of cigarettes or ≥360 g of tobacco in their lifetime.13–19 Former smokers at baseline or follow-up were smokers who had quit smoking at least 1 month before the examination. Current smokers reported active smoking at the interview. The amount of cigarette exposure of participants was assessed by pack-years. Environmental tobacco smoke (ETS) was defined by a positive answer to the question: “Have you been regularly exposed to tobacco smoke in the last 12 months? (‘regularly’ means on most days or nights)?”.

**Genotyping**

Whole blood was sampled at follow-up and DNA was extracted manually19 using the Gentra Puregene Kit (Gentra Systems, Minneapolis, USA). Genotyping of the GSTP1 Ile105Val single nucleotide polymorphism and the gene deletion polymorphisms for GSTM1 and GSTT1 was performed by TaqMan methodology as previously described.22 Hardy-Weinberg equilibrium (HWE) for the GSTP1 Ile105Val polymorphism was tested using Arlequin Version 2,00020 and genotype distribution was found to be in HWE. Genotype frequencies did not differ by Swiss language region or subjects’ nationality.

**Study sample**

We included in the present analysis SAPALDIA participants with valid spirometric and bronchial challenge data from the baseline examination and questionnaire data who participated in the interview at follow-up (n = 5825; see fig 1 in online supplement). Valid information on respiratory health status at baseline and follow-up as well as genotype information on the GST polymorphisms were available for 4682 of these participants. Subjects who had reported physician-diagnosed asthma at baseline were excluded (n = 256). The final sample size for this study consisted of 4426 SAPALDIA participants. Missing information for the covariates baseline forced vital capacity (FVC) (n = 105), atopy (n = 99), pack-years smoked during follow-up (n = 112), ETS exposure (n = 5) and occupational inhalant exposure (n = 532) reduced the study sample available for multiple regression analysis to 3806 subjects, of which 3.1% reported physician-diagnosed asthma for the first time at follow-up (n = 119).

**Statistical analysis**

Differences in genotype frequency between cases and controls were assessed by χ² test. The associations between GST genotypes and bronchoconstrictor response slope measured as the percentage fall in FEV1 per μmol methacholine at baseline were assessed by multiple linear regression adjusting for baseline information on FEV1, height, weight, age, BMI, sex, smoking status and pack-years smoked; predicted values for GSTP1 Ile105Val genotypes were displayed using the box plot command. The effects of BHR at baseline and GST genotypes on the risk for reporting new physician-diagnosed asthma and current asthma symptoms at follow-up were estimated using multiple logistic regression analysis adjusted for sex, age, baseline measures of FVC and BMI, weight change during follow-up, and follow-up information on smoking status, amount of pack-years smoked, and exposure to ETS, occupational dust, fumes and vapours. Modification of the effect of baseline BHR on incident asthma reports by GSTP1 Ile105Val genotype was assessed by including a multiplicative interaction term in the model, as well as by stratifying the analysis by genotype. Cumulative asthma incidences with 95% confidence intervals (CI) in subgroups defined by combinations of GSTP1 Ile105Val genotypes and baseline BHR were calculated using the adjust command after multivariate logistic regression analysis. Two-sided p values of <0.05 and <0.10 were considered statistically significant for main effects and interactions.26 All analyses were conducted using STATA SE Version 9.1 (Stata Corporation, Texas, USA).

**RESULTS**

The characteristics of the study population overall and stratified by the presence or absence of baseline BHR are shown in table 1. Among the 4426 participants free of asthma at baseline, 3.3% (n = 144) reported a history of physician-diagnosed asthma at the follow-up examination.

Of the study sample, 14% had BHR at baseline; women were overrepresented in the group exhibiting BHR. Lung function was lower and the proportion of subjects with FEV1/FVC <0.70 was higher in the subgroup with BHR. Smokers were more likely to have BHR and the mean amount of pack-years smoked was higher in subjects with BHR, whereas no difference in ETS exposure or occupational exposure to gas, dust and fumes was noted between the two BHR groups. Baseline respiratory symptoms such as wheezing, chronic cough and phlegm and shortness of breath at night were more prevalent in the group with BHR; 19.7% of subjects with BHR reported at least one respiratory symptom compared with only 11.5% of participants.
without BHR. The GST genotype distributions agreed well with those previously reported in other Caucasian populations.13 16 28 Earlier reports suggested that GSTP1 genotype might be associated with BHR.16 Table 1 shows that the distribution of the various GSTP1 Ile105Val genotypes was not materially different among participants with or without BHR. Homozygous Ile carriers were slightly underrepresented among subjects with baseline BHR but the difference did not reach statistical significance (44.6% vs 47.2%, p = 0.23; table 1). We also compared the adjusted methacholine dose response according to GST genotypes (fig 1). No difference in response to the bronchoconstrictor was observed between the three GSTP1 Ile105Val genotypes. GSTM1 and GSTT1 genotypes were not associated with response to methacholine (data not shown). Homozygous GSTP1 Ile/Ile genotypes were slightly overrepresented among subjects with asthma at baseline (p = 0.06). Neither GSTM1 nor GSTT1 genotype was associated with self-reported physician-diagnosed asthma at baseline (see table 1 in online supplement).

Table 1 Characteristics* of the study population overall and stratified by the presence or absence of baseline BHR

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All</th>
<th>Without BHR</th>
<th>With BHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort participants included in study, N (%)</td>
<td>4426 (100)</td>
<td>3800 (85.9)</td>
<td>626 (14.1)</td>
</tr>
<tr>
<td>Women, N (%)</td>
<td>2169 (49.0)</td>
<td>1758 (46.3)</td>
<td>411 (66.7)</td>
</tr>
</tbody>
</table>

Baseline characteristics

| Mean (SD) age at baseline (years)                   | 40.5 (11.3) | 40.3 (11.2) | 41.4 (11.7) |
| BMI at baseline (kg/m²)                             | 23.7 (3.5)  | 23.6 (3.4)  | 23.9 (4.0)  |
| Total IgE >100 kU/l, N (%)                          | 821 (20.1)  | 657 (18.6)  | 164 (29.3)  |
| Atopy, N (%)                                        | 908 (21.0)  | 680 (19.6)  | 159 (28.0)  |
| Geometric mean (SD) methacholine response slope†    | 1.0 (3.7)   | 0.7 (2.9)   | 7.1 (2.8)   |
| Non-smokers, N (%)                                  | 2069 (46.8) | 1760 (46.9) | 289 (46.3)  |
| Former smokers, N (%)                               | 1016 (23.0) | 900 (23.7)  | 116 (18.6)  |
| Current smokers, N (%)                              | 1337 (30.2) | 1118 (29.4) | 219 (35.1)  |
| Mean (SD) pack-years among ever smokers at baseline | 16.5 (17.7) | 16.0 (17.3) | 19.7 (19.6) |
| ETS exposure reported at baseline, N (%)            | 879 (19.9)  | 757 (19.9)  | 122 (18.8)  |
| Gas, fumes and dust exposure at work at baseline, N (%) | 1348 (30.6) | 1144 (30.2) | 204 (32.6)  |

Selected follow-up characteristics

| Mean (SD) FEV₁, % pred | 101.6 (12.2) | 102.7 (11.8) | 94.7 (12.1) |
| Mean (SD) FVC (l)      | 4.6 (1.0)    | 4.7 (1.0)    | 4.2 (1.0)   |
| Mean (SD) FEV₁/FVC (%) | 79.8 (6.9)   | 80.2 (6.6)   | 77.3 (7.2)  |
| FEV₁/FVC <0.7, N (%)   | 324 (7.5)    | 232 (6.3)    | 92 (15.0)   |
| Wheezing without cold in last 12 months, N (%)      | 207 (4.7)    | 149 (3.9)    | 58 (9.3)    |
| Chronic phlegm, N (%)                                    | 222 (5.1)    | 181 (4.8)    | 41 (6.7)    |
| Chronic cough, N (%)                                      | 157 (3.6)    | 119 (3.1)    | 38 (6.1)    |
| Never smokers, N (%)                                      | 2069 (46.8)  | 1792 (47.2)  | 253 (44.6)  |
| Ever smokers, N (%)                                       | 1905 (43.0)  | 1631 (42.8)  | 267 (45.0)  |
| Mean (SD) pack-years†                                     | 2521 (57.0)  | 2164 (57.0)  | 357 (57.0)  |
| ETS exposure reported at follow-up, N (%)               | 879 (19.9)   | 757 (19.9)   | 122 (18.8)  |
| Gas, fumes and dust exposure at work at follow-up, N (%) | 1348 (30.6)  | 1144 (30.2)  | 204 (32.6)  |

GST genotypes

<table>
<thead>
<tr>
<th>GSTP1 Ile105Val, N (%)</th>
<th>Ile/Ile</th>
<th>2071 (46.8)</th>
<th>1792 (47.2)</th>
<th>253 (44.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile/Val</td>
<td>1945 (43.9)</td>
<td>1650 (43.4)</td>
<td>266 (46.9)</td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>410 (9.3)</td>
<td>358 (9.4)</td>
<td>48 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Ile/Val or Val/Val</td>
<td>2355 (52.5)</td>
<td>1828 (52.5)</td>
<td>314 (55.4)</td>
<td></td>
</tr>
<tr>
<td>GSTM1, N (%)</td>
<td>No homozygous deletion</td>
<td>2100 (47.5)</td>
<td>1802 (47.5)</td>
<td>298 (47.7)</td>
</tr>
<tr>
<td>Homozygous deletion</td>
<td>2322 (52.5)</td>
<td>1995 (52.5)</td>
<td>327 (52.3)</td>
<td></td>
</tr>
<tr>
<td>GSTT1, N (%)</td>
<td>No homozygous deletion</td>
<td>3600 (81.4)</td>
<td>3090 (81.4)</td>
<td>510 (81.6)</td>
</tr>
<tr>
<td>Homozygous deletion</td>
<td>822 (18.6)</td>
<td>707 (18.6)</td>
<td>115 (18.4)</td>
<td></td>
</tr>
</tbody>
</table>

BHR, bronchial hyperresponsiveness; BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; ETS, environmental tobacco smoke; GST, glutathione S-transferase.

*Given as absolute numbers and percentages for categorical variables and as mean (SD) for continuous variables.

†Numbers do not consistently add up to full sample size owing to missing information on some of the presented characteristics.

‡Atopy was defined as a positive skin prick test reaction to at least one common allergen.

§Percentage decrease in FEV₁ per μmol methacholine challenge

*Pack years smoked during follow-up among ever smokers.

Asthma


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In table 2 we present the unadjusted association of both GST genotypes and baseline BHR with physician-diagnosed asthma at follow-up. Both baseline BHR and GSTP1 Ile105Val genotype—but not GSTM1 or GSTT1 gene deletion polymorphisms—were associated with new asthma reports at follow-up (p < 0.001 for baseline BHR and p = 0.005 for GSTP1 genotype). The independent associations of baseline BHR and GSTP1 genotype with physician-diagnosed asthma at follow-up were confirmed after adjustment for potential confounders and mutual adjustment for each other (fig 2). The adjusted odds ratio (OR) for BHR vs no BHR was 3.52 (95% CI 2.31 to 5.35; p < 0.001) and for GSTP1 105Ile/Ile vs GSTP1 105Val genotypes it was 1.71 (95% CI 1.17 to 2.50; p = 0.006).

The BHR effect on new reports of asthma and asthma symptoms was modified by GSTP1 (table 3). The effect of baseline BHR on the risk for new reports of asthma was stronger for the GSTP1 Ile/Ile genotype (OR 4.84 (95% CI 2.28 to 8.49) vs 2.42 (95% CI 1.25 to 4.67); p for interaction between BHR and GSTP1 Ile105Val genotype pBHR*GSTP1 = 0.117). The effect modification by GSTP1 genotype reached statistical significance after exclusion of subjects reporting at least one respiratory symptom at baseline (OR 15.18 (95% CI 5.32 to 47.26) vs 1.95 (95% CI 0.71 to 6.96); pBHR*GSTP1 = 0.033). No modification of the BHR/asthma association by GSTT1 or GSTM1 gene deletion was observed (data not shown).

The adjusted 11-year cumulative incidence of self-reported physician-diagnosed asthma was 14 cases per 1000 persons (95% CI 9 to 21) among subjects without baseline BHR and no GSTP1 Ile/Ile genotype; 19 cases per 1000 (95% CI 13 to 28) among subjects without BHR but with GSTP1 Ile/Ile genotype; 21 cases per 1000 (95% CI 9 to 43) among subjects with BHR and no GSTP1 Ile/Ile genotype; and 95 cases per 1000 (95% CI 59 to 150) among subjects with both baseline BHR and GSTP1 105Ile/Ile genotype. Thus, among participants with baseline BHR in this study population, the excess number of incident asthma cases due to GSTP1 Ile/Ile genotype was 74/1000 persons over the 11-year follow-up period.

Since GST genotypes have previously been associated with lung growth in children and with lung function in adults, we performed a sensitivity analysis and omitted the adjustment for baseline lung function from all statistical models. None of the effects presented were materially altered (data not shown).

### Table 2 Unadjusted association of baseline BHR and GST genotypes with self-report of physician-diagnosed asthma after 11 years of follow-up

<table>
<thead>
<tr>
<th>Subjects at risk</th>
<th>New asthma reports</th>
<th>p Value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline BHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without BHR</td>
<td>3600</td>
<td>94/2.5%</td>
</tr>
<tr>
<td>With BHR</td>
<td>626</td>
<td>50/8.0%</td>
</tr>
<tr>
<td>GSTP1 Ile105Val</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile/Val or Val/Val</td>
<td>2355</td>
<td>60/2.6%</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>2071</td>
<td>77/4.0%</td>
</tr>
<tr>
<td>GSTM1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No deletion</td>
<td>2100</td>
<td>71/3.4%</td>
</tr>
<tr>
<td>Deletion</td>
<td>2322</td>
<td>73/3.1%</td>
</tr>
<tr>
<td>GSTT1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No deletion</td>
<td>3600</td>
<td>119/3.3%</td>
</tr>
<tr>
<td>Deletion</td>
<td>822</td>
<td>25/3.0%</td>
</tr>
</tbody>
</table>

BHR, bronchial hyperresponsiveness; GST, glutathione S-transferase.
Asthma

Table 3  Adjusted risk* for self-reported physician-diagnosed asthma and asthma symptoms at follow-up in relation to bronchial hyperresponsiveness (BHR) at baseline, stratified by GSTP1 Ile105Val genotype

<table>
<thead>
<tr>
<th>GSTP1 Ile/Ile</th>
<th>Subjects at risk</th>
<th>Cases</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>GSTP1 Ile/Val or Val/Val</th>
<th>Subjects at risk</th>
<th>Cases</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physician-diagnosed asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without BHR</td>
<td>1551</td>
<td>42</td>
<td></td>
<td></td>
<td>1729</td>
<td>35</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With BHR</td>
<td>232</td>
<td>27</td>
<td>4.84 (2.28 to 8.49)</td>
<td>&lt;0.001</td>
<td>294</td>
<td>15</td>
<td>2.42 (1.25 to 4.67)</td>
<td>0.009</td>
<td>0.117</td>
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<tr>
<td>Current asthma symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without BHR</td>
<td>1551</td>
<td>10</td>
<td>1</td>
<td></td>
<td>1729</td>
<td>13</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With BHR</td>
<td>232</td>
<td>16</td>
<td>14.7 (5.76 to 37.36)</td>
<td>&lt;0.001</td>
<td>294</td>
<td>9</td>
<td>4.06 (1.59 to 10.40)</td>
<td>0.003</td>
<td>0.115</td>
</tr>
</tbody>
</table>

After exclusion of patients with symptoms at baseline†

<table>
<thead>
<tr>
<th>Physician-diagnosed asthma</th>
<th>Subjects at risk</th>
<th>Cases</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>GSTP1 Ile/Val or Val/Val</th>
<th>Subjects at risk</th>
<th>Cases</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without BHR</td>
<td>1384</td>
<td>37</td>
<td>1</td>
<td></td>
<td>1524</td>
<td>30</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With BHR</td>
<td>186</td>
<td>21</td>
<td>4.57 (2.43 to 8.57)</td>
<td>&lt;0.001</td>
<td>236</td>
<td>7</td>
<td>1.40 (0.58 to 3.39)</td>
<td>0.45</td>
<td>0.023</td>
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<tr>
<td>Current asthma symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without BHR</td>
<td>1384</td>
<td>9</td>
<td>1</td>
<td></td>
<td>1524</td>
<td>12</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With BHR</td>
<td>186</td>
<td>11</td>
<td>15.86 (5.32 to 47.26)</td>
<td>&lt;0.001</td>
<td>236</td>
<td>4</td>
<td>1.95 (0.71 to 6.96)</td>
<td>0.29</td>
<td>0.033</td>
</tr>
</tbody>
</table>

*Adjusted for baseline measures of forced vital capacity and body mass index, weight change during follow-up, sex, age, smoking status (never/persistent/others), pack-years smoked to follow-up, environmental tobacco smoke (follow-up) and occupational dust/fumes/vapour exposure (follow-up), study area.
†Subjects excluded (n = 476) who reported at baseline at least one of the following respiratory symptoms: wheezing without a cold; chronic cough; chronic phlegm; woken up by shortness of breath at night.
‡p Values for interaction between bronchial hyperresponsiveness (BHR) and GSTP1 Ile105Val genotype obtained by including multiplicative interaction term in multiple logistic regression analysis.

DISCUSSION

We present evidence that adults with BHR and GSTP1 Ile/Ile genotype are at an increased risk of developing asthma. Furthermore, genetic variation in GSTP1 (but not GSTM1 or GSTT1) modifies the risk of BHR progression to asthma in the general population. As GSTP1 exhibits the highest lung tissue expression of the three GST enzymes, our results are in line with the hypothesis that the extent of local inflammation in the airways contributes to airway remodelling and thus to the association between BHR and adult onset asthma.

Airway smooth muscle dysfunction30–31 and increased airway wall thickening32–33 caused by airway remodelling are commonly thought to underlie BHR and possibly its progression to asthma. Prolonged or repeated exposure to airway irritants (such as tobacco smoke, ambient air pollution or occupational activity-derived particles) can cause airway dysfunction of the smooth muscle and the bronchial epithelium through the induction of chronic airway inflammation,31 34 35 BHR itself contributes to a harmful cycle of sustained inflammation since the associated abnormal airflow resulting from the reduced airway calibre alters the deposition profile of inhalants in the airways.36–38 It therefore seems probable that increased depositions of particles with mostly oxidative properties will result in sustained airway inflammation and oxidative stress. This view is corroborated by recent SAPALDIA results showing that BHR increased the effect of ETS exposure on the incidence of asthma-related symptoms.3 40

According to our results, the genetic make-up of a person co-determines the exposure load necessary to induce sustained inflammation, oxidative stress and airway remodelling in the bronchial tissue. The modifying effect of the GSTP1 Ile105Val genotype may reflect the different (but related) roles of this enzyme (ie, in phase II detoxification of tobacco- or air pollution-derived chemicals,39 in oxidant defence41 and in cell cycle regulation42). Lower GSTP1 activity in bronchial tissue is likely to result in decreased detoxification of airway irritants, enhanced inflammation and oxidative stress causing sustained airway wall thickening and smooth muscle dysfunction. This hypothesised pathophysiological mechanism is supported by evidence for the direct involvement of GSTP1 in the regulation of C-JUN N-terminal protein kinase (JNK) and downstream processes which lead to increased cell proliferation in response to oxidative stress.43 44 Mice deficient for GSTP1 activity are prone to increased tumorigenesis on exposure to polycyclic hydrocarbons.45 The modifying effect of GSTP1 in the BHR/asthma association may therefore result from the central role of this enzyme and oxidative stress in apoptotic processes. Passive smoking has been shown to confer increased oxidative stress locally in bronchial tissue as well as systemically in exposed subjects.46–48 Second, obesity characterised by increased systemic inflammation has been associated with increased progression of BHR and asthma.31 Third, Cheng et al32 found a gradient of free radical concentrations in nasal polyps, an inflammatory chronic disease frequently associated with BHR and asthma. Concentrations were lowest in cases with nasal polyps without BHR and asthma, intermediate in those with concurrent silent BHR and highest in cases of nasal polyps with concurrent BHR and asthma. Genetic GST variants have previously been associated with asthma in different, exclusively cross-sectional, studies. The prevalence of the GSTP1 105Val allele was lower in patients with asthma,31 32 BHR46 and asthmatics with severe disease.14 17 39 54 Our findings extend this observation and indicate that the GSTP1 105Ile/le genotype, which is carried by about 47% of our European Caucasian population, represents a prevalent asthma risk factor in the general adult population. The absence of a cross-sectional GSTP1/BHR association in our population-based study contrasts with the positive association found by Fryer and colleagues.50

50 found a...
the SAPALDIA cohort, the GSTP1/BHR association was not modified by gender (data not shown). Our finding of a lack of association between GSTM1 and GSTT1 gene deletion and the occurrence of asthma also contrasts with some previous results. A few small case-control studies suggest that the prevalence of GSTM1 and GSTT1 gene deletions is increased in patients with asthma with concurrent atopy.59 60 In a limited number of cohorts, GSTM1 and GSTT1 deficiency increased the risk for asthma, asthma-related symptoms and low lung function during childhood in combination with in utero and/or current passive smoking exposure.61 62 Protective effects of antioxidant supplementation on the lung function of children with asthma exposed to high ambient ozone were restricted to GSTM1-deficient participants.63 The discrepancy between our study and previous reports with regard to the role of GSTM1 and GSTT1 deletions might partly be related to differences in study population, sample size, study design or inhalant exposures. Most importantly, we focused on adult incident asthma whereas the evidence for an association between asthma and GSTM1 or GSTT1 was most consistently found in children. Unfortunately, our sample size was insufficient to additionally stratify the results by atopy or exposure to environmental inhalants including ETS.

The strength of the present study is its prospective design which allowed us to investigate the long-term effects of BHR in the general population. The large sample size of the cohort and its detailed characterization made it possible to investigate the interaction between BHR and GST genotypes on the incidence of adult onset asthma. In addition, it was possible to refine the analysis after exclusion of participants who might have had asthma symptoms at baseline without the formal diagnosis of asthma. Nevertheless, the number of new asthma cases available to estimate the effect modification of baseline BHR by the GSTP1 genotype was limited. Independent studies are therefore needed to confirm this novel finding.

The definition of asthma in the present study relied solely on the self-report of asthma phenotypes. Asthma status may therefore be subject to misclassification.59 60 The unknown degree of population stratification is another limitation, as no panel of anonymous markers was tested to assay the population admixture of the Swiss general population. However, stratification of the associations by study centre, language region and nationality did not materially alter the main findings, suggesting only a minor influence of population stratification at baseline. We cannot exclude participation bias. Participants at baseline were less likely to be of intermediate age or of Swiss nationality, but were more likely to be former smokers and to report asthma or wheezing than non-participants.15 19 Unlike participation at baseline, patients with asthma were less likely to participate at follow-up.3 19 Among the non-participants at the follow-up examination there were slightly more men, smokers, subjects with low educational background, subjects with occupational exposure to fumes, gas and dust, and with respiratory symptoms. No difference was noted for atopy and BHR between participants and non-participants at follow-up. Subjects with low lung function— a risk factor for BHR— were less likely to undergo methacholine challenge,3 but we had previously presented results from the SAPALDIA cohort which showed that, in contrast to previous evidence,15 GSTP1 was not associated with lung function in this adult sample from the general population.19 In the absence of genotype information on non-participants at baseline and follow-up, we cannot analyse whether we failed to include subjects with unique genotype/phenotype combinations. Even though it is unlikely that genotype status influenced participation, this possible source of bias also points to the need for independent studies to confirm our result.

In conclusion, we present evidence that the GSTP1 Ile/Ile genotype may be a strong and prevalent risk factor for the progression of silent BHR to asthma in the general population. If confirmed by additional studies, the results are consistent with the hypothesis that genetic variation in the local metabolism of inhalant-derived chemicals and/or free radicals plays a relevant role in the progression from BHR to asthma.

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SAPALDIA Team: Study directorate: T Rochat (p), U Ackermann-Liebrich (e), J M Gaspoz (c), P Leuenberger (p), LJ Liu (exp), NM Probst Hensch (e/g), C Schindler (s).

Scientific team: J C Barthélémé (c), W Berger (g), R Betschart (p), A Bircher (a), G Bolognini (p), O Brändli (p), M Brutsche (p), L Burdet (p), M Frey (p), MW Gerbase (p), D Gold (e/c/p), W Karrer (p), R Keller (p), KN Kötzli (e/exp), U Neu (exp), L Nicod (p), M Pons (p), E Russi (p), P Schmid-Grendelmeier (a), J Schwartz (e), P Straehl (exp), JM Tschopp (p), A von Eckardstein (cc), J P Zellweger (p), E Zemp Stutz (e).

Scientific team at coordinating centres: P O Briveaux (p), I Curjuric (e), S H Downs (e/s), D Felber Dietrich (c), A Gempfer (s), D Kedel (s), M Imboden (p), P Staedele-Kessler (s), G A Thun (g), I allergology, (c) cardiology, (cc) clinical chemistry, (e) epidemiology, exp exposure, (g) genetic and molecular biology, (m) meteorology, (p) pneumology, (s) statistics.

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M Imboden, T Rochat, M Brutsche, C Schindler, S H Downs, M W Gerbase, W Berger, N M Probst-Hensch and the SAPALDIA Team

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