Glutathione-S-transferase genes and asthma phenotypes: a Human Genome Epidemiology (HuGE) systematic review and meta-analysis including unpublished data

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Background Oxidative stress is thought to be involved in the pathogenesis of asthma. Glutathione-S-transferase (GST) enzymes, which play an important role in antioxidant defences, may therefore influence asthma risk. Two common deletion polymorphisms of GSTM1 and GSTT1 genes and the GSTP1 Ile105Val polymorphism have been associated with asthma in children and adults, but results are inconsistent across studies.

Methods Systematic review and meta-analysis of the effects of GST genes on asthma, wheezing and bronchial hyper-responsiveness (BHR), with inclusion of unpublished data from three studies, including the large Avon Longitudinal Study of Parents and Children (ALSPAC). Random effect or fixed effect models were used as appropriate, and sensitivity analyses were performed to assess the impact of study characteristics and quality on pooled results.

Results The meta-analyses of GSTM1 (n = 22 studies) and GSTT1 (n = 19) showed increased asthma risk associated with the null genotype, but there was extreme between-study heterogeneity and publication bias and the association disappeared when meta-analysis was restricted to the largest studies. Meta-analysis of GSTP1 Ile105Val (n = 17) and asthma suggested a possible protective effect of the Val allele, but heterogeneity was extreme. Few studies evaluated wheezing and BHR and most reported no associations, although weak evidence was found for positive associations of GSTM1 null

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Conclusions Our findings do not support a substantial role of GST genes alone in the development of asthma. Future studies of large size should focus on interactions of GST genes with environmental oxidative exposures and with other genes involved in antioxidant pathways. Quality of study conduct and reporting needs to be improved to increase credibility of the evidence accumulating over time.

Keywords Meta-analysis, systematic review, glutathione-S-transferase genes, GSTM1 gene, GSTT1 gene, GSTP1 gene, asthma, wheezing, bronchial responsiveness, The Avon Longitudinal Study of Parents and Children (ALSPAC)

Introduction
Asthma is characterized by chronic airway inflammation and oxidative stress in the lungs has been implicated in its pathogenesis. Sources of oxidant injury are reactive oxygen and nitrogen species generated by activated inflammatory cells and bronchial epithelial cells and inhalation of atmospheric pollutants, notably tobacco smoke and oxidant gases, including ozone, sulphur dioxide and nitrogen oxides. These are countered by enzymatic and non-enzymatic antioxidants, including dietary antioxidants, such as vitamins C and E, and glutathione, a major protective antioxidant in the lungs that also has a role in regulation of inflammatory responses. A family of enzymes, the glutathione-S-transferases (GSTs), has the general function of conjugating glutathione with electrophilic substances that are capable of generating free radicals, thus leading to detoxification of their effects. Genetic polymorphisms associated with reduced activity of GSTs are therefore of interest in the study of disease susceptibility. Two common deletion polymorphisms of GSTM1 and GSTT1 genes have been associated with asthma in children and adults. The Val allele of the GSTP1 Ile105Val polymorphism, although associated with reduced glutathione activity, has surprisingly been reported to be highly protective for asthma and airway responsiveness in adults. However, the Val105 allele has also been reported to increase the risk of asthma and to increase susceptibility to the effects of ozone on breathing difficulties in children with asthma. Others have reported no associations between this GSTP1 polymorphism and asthma in children and adults. Many of the studies were relatively small or confined to selected populations. A systematic review of the literature can help understand the reasons for the heterogeneity in study results, and the pooling of results across similar studies in a meta-analysis can overcome the problem of limited statistical power. With this aim, we have conducted a systematic review and meta-analysis on the associations between GSTM1, GSTT1 and GSTP1 polymorphisms and asthma phenotypes in children and adults, with inclusion of unpublished data from a large UK cohort study in children, a UK family-based study in children and young adults and a Spanish cohort study in children.

Methods
Primary studies
Methods of the three primary studies included in the meta-analyses are briefly summarized below. Further details are reported in the Supplementary Appendix 1 available as supplementary data at IJE online.

ALSPAC
The Avon Longitudinal Study of Parents and Children (ALSPAC) is a longitudinal, population-based birth cohort study that recruited 14,541 pregnant women in 1991–92, with 14,062 liveborn children. Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

The study collected data on asthma and wheezing in children at age 7.5 years, based on a questionnaire sent to mothers. Children from multiple births were excluded from the analyses. Asthma was defined as a positive response to the question ‘Have you ever had asthma?’. Spirometry was performed at 8.5 years, and bronchial hyper-responsiveness (BHR) to methacholine was defined as a 20% reduction from baseline (post-saline) Forced Expired Volume in 1 s (FEV\textsubscript{1}) at a cumulative dose of methacholine $\leq$ 1.2 mg. In the mothers’ population, asthma was defined as a positive response to the question ‘Have you ever had asthma?’.
The study has been described previously.\textsuperscript{18,19} Ethical consent was obtained from the Balearic Islands Research Ethics Committee. Approval was obtained from the Balearic Islands Government (Cáritas) and from the Research Ethics Committee of the University of Valencia. Informed consent was obtained from all participants. Sex, age, and smoking status was included in the study questionnaire. The majority of the children’s DNA samples were extracted from cord blood or venous blood collected at age 7 years with a small number extracted from venous blood collected at 43–61 months. The majority of maternal DNA samples were extracted from blood taken during pregnancy and a small number were buccal DNA extracted from mouthwash samples.\textsuperscript{16} GST\textsubscript{P1} polymorphism Ile105Val was determined using competitive allele specific polymerase chain reaction (PCR) system (KASPar). Copy number variation (CNV) of GST\textsubscript{T1} and GST\textsubscript{M1} deletion polymorphisms was analysed using a real-time PCR method.\textsuperscript{17} Genotyping was performed blind to the outcome status of individuals.

The genetic effects of the three genes were evaluated using between-genotype comparisons (variant homozygotes vs wild homozygotes and heterozygotes vs wild homozygotes). Since the samples of mothers and children were not independent, the odds ratios (ORs) used in the meta-analysis for the two groups were estimated by analysing them within the same logistic regression model, and calculating confidence intervals (CIs) using Huber variances clustered by pregnancy.

**AMICS-INMA study**

The Menorca Asthma Multicentre Infant Cohort Study (AMICS) is a population-based birth cohort included in the Spanish environment and childhood research network (INMA study).\textsuperscript{2,21} In 1997–98, the study recruited 482 children at birth from 492 pregnant women resident in the island of Menorca, Spain. The study has been described previously.\textsuperscript{18,19} Ethical approval was obtained from the Balearic Islands Research Ethics Committee.

Information on asthma and wheezing in children was collected through telephone questionnaires administered yearly to mothers, for children up to the age of 6 years. Presented here are data based on the questionnaire at age 6 years. Asthma was defined as doctor-diagnosis of asthma in the previous 12 months, and wheezing as a positive response to the question ‘Has your child had any wheezing with whistling on his/her chest when he/she breathed in the past 12 months?’. Most of the DNA samples (87%) were extracted from blood obtained at age 4 years, and the rest from saliva collected at age 6 years. GST\textsubscript{M1} and GST\textsubscript{T1} gene deletions were detected by multiplex PCR, with a method modified from Arand and coll\textsuperscript{20} (Supplementary Appendix 1 available as supplementary data at *IJE* online). GST\textsubscript{P1} polymorphism Ile105Val was determined using pyrosequencing (Biotage, Uppsala, Sweden). All assays were performed blind to the outcome status of the children.

GST\textsubscript{M1} and GST\textsubscript{T1} genetic effects were evaluated assuming a recessive model (OR for the null genotype), whereas the genetic effect of GST\textsubscript{P1} was assessed using between-genotype comparisons.

**Southampton study**

The Southampton Asthma Cohort is a family study that started in 1997 and recruited 341 families (1508 individuals) from Southampton, Portsmouth, Bournemouth and the Isle of Wight, UK.\textsuperscript{21} Inclusion criteria required two siblings (5–21 years) diagnosed with asthma and currently using asthma medication. Reported here are the associations of GST\textsubscript{M1} and GST\textsubscript{T1} deletion polymorphisms with asthma, defined as physician’s diagnosis and current use of medication, and bronchial responsiveness to methacholine, only measured in participants with a baseline FEV\textsubscript{1} \textgreater{} 70% predicted.

DNA from blood samples was analysed for CNV of GST\textsubscript{M1} and GST\textsubscript{T1} genes, using quantitative real-time PCR (Supplementary Appendix 1 available as supplementary data at *IJE* online). Genotyping was performed blind to the outcome status.

Data were analysed using Family Based Association Tests (FBAT version 1.4 software; http://www.biostat.harvard.edu/_fbat.htm), assuming an additive genetic model with a null hypothesis of ‘no association and no linkage’ between presence/absence of the gene and asthma phenotypes.\textsuperscript{22}

**Systematic review**

This systematic review was conducted following a protocol in accordance with the Human Genome Epidemiology Network (HuGENet) guidelines. Studies were identified through electronic search of MEDLINE, EMBASE, ISI Web of Science and HuGENet up to February 2008, using a comprehensive search strategy (Supplementary Figure S1 available as supplementary data at *IJE* online). Included were population-based and family-based studies examining the effect of GST polymorphisms on asthma phenotypes. Although the original search strategy included atopy, this article focuses on the association of GST genes with asthma, wheezing and BHR. No restrictions were placed on language and type of report, with inclusion of conference abstracts. When multiple reports were available for a single study, only the most recent article or that with the largest sample size was included. Additional studies were identified through cross-checking of reference lists of all relevant studies, including previous reviews and editorials. Two reviewers (C.M. and R.G.) independently extracted data using a pre-piloted form, with a third reviewer available for arbitration (J.W.H.). Authors of eligible primary studies were contacted for further information whenever the data required by the meta-analysis were not fully reported in the article.

Study quality of primary studies was evaluated based on the HuGENet guidelines\textsuperscript{23} and STrengthening the REporting of Genetic Association studies (STREGA) recommendations for the reporting of genetic association studies.
of genetic association studies. We also assessed the epidemiological credibility of the results of our meta-analyses by use of a index recently proposed for assessing cumulative evidence in genetic associations (Venice criteria), which classifies epidemiological credibility of the results of a meta-analysis as 'strong', 'moderate' or 'weak', based on three elements: (i) amount of evidence, (ii) extent of replication and (iii) protection from bias.

**Meta-analysis**

The meta-analyses were performed on the population-based studies, and included unpublished results from the ALSPAC and AMICS-INMA studies, together with those of studies identified through the systematic review.

Most studies evaluated GSTM1 and GSTT1 as presence/absence of gene deletion, so that meta-analyses of these polymorphisms were performed using a single OR (null vs present). Results for GSTP1 Ile105Val are reported as two ORs, Ile/Val vs Ile/Ile and Val/Val vs Ile/Ile. Given the absence of a priori evidence on the genetic model for this polymorphism, a 'genetic model-free' approach was also used to pool results across genotype groups. This approach does not assume a specific genetic model but estimates it from the data, with the only assumption that the unknown genetic model is constant across studies. The genetic model-free approach was implemented within a Bayesian framework and assuming non-informative prior distributions for all parameters. Details of the model are reported in the Appendix 1.

In the presence of at least five studies, meta-analyses were performed using random effects models, which account for between-study heterogeneity. If less than five studies were included, meta-analysis was performed only in the presence of small between-study heterogeneity ($I^2 < 25\%$), using fixed-effect models. The presence of between-study heterogeneity was investigated using the Q test and its magnitude estimated using the $I^2$ statistic, which measures the proportion of variation across studies due to genuine differences rather than random error. Possible causes of heterogeneity were investigated by subgroup analyses based on geographic location, population age (adults vs children), size of the study and definition of asthma. Additional sensitivity analyses were performed by excluding studies with poor methodological quality. Studies were considered of poor quality if no definition of the disease outcome was provided and either the description of the study sample (e.g. selection of cases/controls for case-control studies) was incomplete or there was no mention of Hardy-Weinberg equilibrium (HWE). Although many more items were considered in the quality assessment, we chose to limit exclusion to those studies where poor quality seemed beyond any reasonable doubt.

For each study, deviation from HWE was tested using the exact test, and the magnitude of the departure measured using the disequilibrium coefficient. Studies with large deviations from HWE were further investigated for possible methodological problems, including population stratification and genotyping errors. The presence of small-study bias, which is a proxy for publication bias, was investigated both graphically using funnel plots and formally using Begg’s and Egger’s tests.

All analyses were performed using Stata for Windows v10 (Stata Corp, College Station, TX, USA), with the exception of the model-free approach, which was implemented in WinBUGS 1.4. For the Bayesian analyses, 95% credible intervals (i.e. range in which the probability that the parameter lies within this interval is 95%) were calculated in place of 95% CIs.

**Results**

**Primary studies**

**ALSPAC**

For analyses on asthma in children, data were available from 5327, 5300 and 5330 children for GSTM1, GSTT1 and GSTP1, respectively; corresponding figures for wheezing were 5991, 5957 and 5992, and for bronchial responsiveness were 3430, 3402 and 3417. For analyses on asthma in mothers, data were available from 7049, 7017 and 7262, respectively. The allele frequencies of GSTM1, GSTT1 and GSTP1 were similar to those reported in other UK populations and in a White, non-Hispanic US population. All gene polymorphisms were in HWE. Overall, there was no evidence of substantial association of the three genes with asthma phenotypes. The results for GSTM1 and GSTT1, analysed as CNV with three-level genotype data, showed no association for GSTM1, whereas a possible small protective effect on asthma of the GSTT1 null allele could not be excluded in mothers (OR: 0.71; 95% CI: 0.57–0.90 and 0.84; 0.63–1.12, for heterozygotes and null homozygotes, respectively, compared with wild-type homozygotes). However, evidence of a possible protective effect was weak in children (0.91; 0.76–1.11 and 0.89; 0.70–1.13). In children, the OR of GSTP1 Ile/Val vs Ile/Ile for asthma was 1.16 (95% CI 0.98–1.37) and for wheezing 1.25 (1.05–1.49), but such association was not shown for GSTP1 Val/Val vs Ile/Ile, and the finding was not replicated in mothers. Most subjects in both children’s and mothers’ groups were White (96 and 98%, respectively), and results did not change when excluding non-White subjects from the analyses.

**AMICS-INMA study**

Data were available from 411 and 404 children for analyses on asthma and wheezing, respectively. GSTP1 genotype was in HWE. No association was found between GSTM1 or GSTT1 and asthma or
wheezing (Figures 2, 3 and 5). GSTP1 showed no association with asthma, but was associated with wheezing (Figures 4 and 5). The ORs for GSTP1 and wheezing were 2.32 (1.03–5.23) and 5.01 (1.72–14.58) for Ile/Val and Val/Val vs Ile/Ile, respectively, which suggests that the increased risk of wheezing associated with the Val allele might follow an additive genetic model.

Southampton study
Among the 341 families, the analyses on asthma included 199 and 224 informative families for GSTM1 and GSTT1, respectively; these figures were 206 and 227 for bronchial responsiveness. No association was found for asthma or bronchial responsiveness with either GSTM1 or GSTT1, although the presence of GSTT1 null allele was associated with an increased severity score in patients with asthma (P = 0.015).

Systematic review and meta-analysis
The process of inclusion and exclusion of studies is presented in Figure 1. Our initial search strategy identified 804 articles, among which the full text of 87 articles was retrieved for more detailed evaluation. Of the 30 eligible articles, 2 reported on family-based studies and 28 articles reported on 26 population-based studies. We contacted authors of 12 articles for further information on genotype counts and outcome definition, and received replies from 5.

Of the 26 eligible population-based studies, we could include in the meta-analyses data from 25 studies.

**Figure 1** Flow chart of the inclusion and exclusion of published articles in the review
(reported in 27 articles), which included 2 cohorts, 2 cross-sectional and 21 case–control studies. After inclusion of the three primary studies (ALSPAC mothers, ALSPAC children, AMICS-INMA) association with asthma was evaluated in 22 studies on GSTM1, 19 on GSTT1 and 17 on GSTP1. Asthma was defined as doctor-diagnosed asthma in 19, 16 and 12 studies on GSTM1, GSTT1 and GSTP1, respectively. Wheezing was evaluated in four studies on GSTM1, two on GSTT1 and three on GSTP1. BHR, defined in all studies as a FEV₁ reduction of ≥20% after methacholine challenge, was considered in three studies on GSTM1, three on GSTT1 and three on GSTP1. Overall, 10 studies were performed in children, 7 in adults, 6 in both, whereas for 2 studies the information was not available. In terms of geographical location, 13 studies were performed in Asia, 8 in Europe, 2 in North Africa and 2 in North America. Case–control studies varied in sample size, from less than 100 to approximately 1000. On the other hand, both cross-sectional studies had around 3000 subjects and three out of five cohort studies had large sample size of 4400–7200. Other characteristics of the population-based studies included in the meta-analyses are summarized in Table 1, and genotype counts by disease outcome are reported in Table 2.

Two family studies were included in the systematic review (Table 1), but only one provided complete Table 1 Characteristics of the studies evaluating the effects of GST genes on asthma risk, wheezing and BHR

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Study population</th>
<th>Location, ethnicity</th>
<th>Adults/children</th>
<th>Sample size</th>
<th>Definition of disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fryer, 2000⁹</td>
<td>Cases: from patient database Controls: healthy volunteers</td>
<td>UK, European</td>
<td>Adults 171</td>
<td></td>
<td>Atopic asthma diagnosed by a physician [Criteria: a history of wheezing, cough, dyspnoea and/or chest tightness; spirometric demonstration of airflow obstruction reversible with ab-agonist bronchodilator (&gt;15% change in FEV₁); and positive atopic status] BHR FEV₁ reduction &gt;20% after methacholine challenge</td>
</tr>
<tr>
<td>Chung, 2002⁵⁹</td>
<td>Cases and controls: from cohort of civil servants and their families</td>
<td>Korea, Asian</td>
<td>N/A 99</td>
<td></td>
<td>Asthma diagnosed by a physician (Criteria: symptoms of wheezing/ chest tightness and FEV₁ reduction &gt;20% after methacholine challenge)</td>
</tr>
<tr>
<td>Freidin, 2002⁶⁰</td>
<td>Cases: hospitalized patients Controls: healthy volunteers</td>
<td>Russia, Asian</td>
<td>N/A 126</td>
<td></td>
<td>Atopic asthma diagnosed by a physician (Criteria: N/A)</td>
</tr>
<tr>
<td>Sideleva, 2002⁶¹</td>
<td>Cases: hospitalized patients Controls: healthy volunteers</td>
<td>Russia, European</td>
<td>Both 199</td>
<td></td>
<td>Atopic asthma diagnosed by a physician (Criteria: the commonly accepted clinical laboratory examination and analysis of the external respiration function)</td>
</tr>
<tr>
<td>Vavilin, 2002;³⁹ Safronova, 2003³⁸</td>
<td>Cases: hospitalized patients Controls: healthy volunteers</td>
<td>Russia, European</td>
<td>Children 237</td>
<td></td>
<td>Asthma diagnosed by a physician (Criteria: N/A)</td>
</tr>
<tr>
<td>Aynacioglu, 2004⁴¹</td>
<td>Cases: consecutive asthma outpatients Controls: healthy volunteers</td>
<td>Turkey, Asian</td>
<td>Adults 475</td>
<td></td>
<td>Asthma diagnosed by a physician (Criteria: ECRHS protocol; medical history, physical examination, lung function tests, chest X-rays, SPT, TOT IgE)</td>
</tr>
<tr>
<td>Saadat, 2004⁶</td>
<td>Cases: N/A Controls: healthy volunteers</td>
<td>Iran, Asian</td>
<td>Both 170</td>
<td></td>
<td>Asthma diagnosed by a physician [Yes to two or more criteria: (i) history of wheezing/breathlessness; (ii) airflow obstruction of &gt;15% FEV₁; and (iii) PEF &gt;20%]</td>
</tr>
<tr>
<td>Tamer, 2004¹¹</td>
<td>Cases: hospitalized patients Controls: healthy volunteers</td>
<td>Turkey, Asian</td>
<td>Adults 204</td>
<td></td>
<td>Asthma diagnosed by a physician (Criteria: American Thoracic Society statement)</td>
</tr>
<tr>
<td>Zhang, 2004³⁶</td>
<td>Cases: hospitalized patients Controls: N/A</td>
<td>China, Asian</td>
<td>Adults 120</td>
<td></td>
<td>Asthma diagnosed by a physician (Criteria: N/A)</td>
</tr>
</tbody>
</table>

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Table 1 Continued

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Study population</th>
<th>Location, ethnicity</th>
<th>Adults/children</th>
<th>Sample size</th>
<th>Definition of disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lee,</em> 2005, 200810,64</td>
<td>Cases: school children from three communities Controls: healthy random sample from same three communities</td>
<td>Taiwan, Asian</td>
<td>Children</td>
<td>397</td>
<td><strong>Asthma</strong> parent-reported (positive response to the question, ‘Has a physician ever diagnosed your child as having asthma?’) <strong>Wheezing</strong> parent-reported (lifetime history)</td>
</tr>
<tr>
<td><em>Nickel,</em> 200513</td>
<td>Cases: from Multicenter Allergy Study Controls: healthy volunteers</td>
<td>Germany, European</td>
<td>Children</td>
<td>205</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: one or more episodes of wheezing during previous 12 months)</td>
</tr>
<tr>
<td><em>Oh,</em> 200537</td>
<td>Cases: recruited at an allergy clinic Controls: healthy subjects</td>
<td>Korea, Asian</td>
<td>Both</td>
<td>273</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: N/A)</td>
</tr>
<tr>
<td><em>Arbag,</em> 200665</td>
<td>Cases: cases of nasal polyposis Controls: healthy individuals from same geographic location and same ethnicity as cases</td>
<td>Turkey, Asian</td>
<td>Both</td>
<td>133</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: history of intermittent wheezing and the presence of reversible airway obstruction as defined by at least a 12% improvement in FEV1, following bronchodilator administration, therapeutic response to anti-asthma treatment, or an abnormal result in methacholine bronchoprovocation test (PG20 &lt;8 mg/dl))</td>
</tr>
<tr>
<td><em>Ercan,</em> 20061</td>
<td>Cases: random sample from cohort of asthmatic children Controls: healthy school children</td>
<td>Turkey, Asian</td>
<td>Children</td>
<td>563</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: history of intermittent wheezing and the presence of reversible airway obstruction as defined by at least a 12% improvement in FEV1, following bronchodilator administration, therapeutic response to anti-asthma treatment, or an abnormal result in methacholine bronchoprovocation test (PG20 &lt;8 mg/dl))</td>
</tr>
<tr>
<td><em>Holla,</em> 200666</td>
<td>Cases and controls are unrelated subjects selected from questionnaires</td>
<td>Czech Republic, European</td>
<td>Both</td>
<td>637</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: asthma symptoms and use of antiasthma medication according to the GINA guidelines)</td>
</tr>
<tr>
<td><em>Plutecka,</em> 200632</td>
<td>Cases: N/A Controls: healthy volunteers</td>
<td>Poland, European</td>
<td>Adults</td>
<td>496</td>
<td><strong>Asthma</strong> not defined</td>
</tr>
<tr>
<td><em>Abdel-Alim,</em> 200740</td>
<td>Cases: children attending outpatient clinic of allergy and immunology Controls: matched healthy children enrolled in this work</td>
<td>Egypt, North African</td>
<td>Children</td>
<td>90</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: GINA guidelines)</td>
</tr>
<tr>
<td><em>Hanene,</em> 20077</td>
<td>Cases and controls from a representative region</td>
<td>Tunisia, North African</td>
<td>Children</td>
<td>347</td>
<td><strong>Asthma</strong> not defined</td>
</tr>
<tr>
<td><em>Kamada,</em> 20077a89</td>
<td>Cases: recruited from a medical centre Controls: healthy volunteers</td>
<td>Japan, Asian</td>
<td>Both</td>
<td>980</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: American Thoracic Society)</td>
</tr>
<tr>
<td><em>Kamada,</em> 20077b89</td>
<td>Cases: recruited from a medical centre Controls: healthy volunteers</td>
<td>Japan, Asian</td>
<td>Children</td>
<td>289</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: American Thoracic Society)</td>
</tr>
<tr>
<td><em>Mak,</em> 20078</td>
<td>Cases: recruited from asthma clinic Controls: random healthy subjects from Hong-Kong population</td>
<td>China, Asian</td>
<td>Adults</td>
<td>626</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: cough/wheeze/chest tightness and airflow obstruction of ≥15% FEV1)</td>
</tr>
</tbody>
</table>

**Cohort studies (n = 5)**

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Study population</th>
<th>Location, ethnicity</th>
<th>Adults/children</th>
<th>Sample size</th>
<th>Definition of disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Imboden,</em> 200771</td>
<td>SAPALDIA—a prospective multicentre study on adult Swiss general population, investigating environmental and genetic effects on lung</td>
<td>Switzerland, European</td>
<td>Adults</td>
<td>4422</td>
<td><strong>Asthma</strong> self-reported (positive response to ‘Have you ever had asthma?’ and ‘Was this confirmed by a doctor?’) <strong>BHR</strong> FEV1 reduction &gt;20% after methacholine</td>
</tr>
</tbody>
</table>

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Table 1 Continued

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Study population</th>
<th>Location, ethnicity</th>
<th>Adults/children</th>
<th>Sample size*</th>
<th>Definition of disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schroer 2009</td>
<td>CCAAPS study on allergy and air pollution in infants with at least one atopic parent</td>
<td>US, mixed ethnicity</td>
<td>Children</td>
<td>498</td>
<td><strong>Wheezeing</strong> parent-reported (parental report of the child wheezing at least once at 2 years in last 12 months)</td>
</tr>
<tr>
<td>ALSPAC mothers</td>
<td>ALSPAC—a UK population-based birth cohort, with pregnant women recruited in 1990</td>
<td>UK, European</td>
<td>Adults</td>
<td>7262</td>
<td><strong>Asthma</strong> self-reported (history of asthma reported in a questionnaire administered during pregnancy)</td>
</tr>
<tr>
<td>ALSPAC children</td>
<td>ALSPAC—children recruited at birth and followed up to investigate their health, behaviour and development</td>
<td>UK, European</td>
<td>Children</td>
<td>5330</td>
<td><strong>Asthma</strong> parent-reported (positive response to ‘Has a doctor ever said that your child has asthma?’ at 7 years)</td>
</tr>
<tr>
<td>AMICS-INMA</td>
<td>AMICS—a population-based birth cohort included in the Spanish environment and childhood research network (INMA study)</td>
<td>Spain, European</td>
<td>Children</td>
<td>428</td>
<td><strong>Asthma</strong> parent-reported (positive response to ‘Has your child had any wheezing with whistling on his/her chest when he/she breathed in the past 12 months?’ at age 6 years)</td>
</tr>
</tbody>
</table>

Cross-sectional studies (n = 2)

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Study population</th>
<th>Location, ethnicity</th>
<th>Adults/children</th>
<th>Sample size*</th>
<th>Definition of disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salam 2007; Gilliland, 2002</td>
<td>Cross-sectional study as part of the Children Health Study, a Californian ongoing cohort study of school children in 12 communities</td>
<td>US, Mixed ethnicity</td>
<td>Children</td>
<td>3081</td>
<td><strong>Asthma</strong> parent-reported DDA (Criteria: N/A)</td>
</tr>
<tr>
<td>Kabesch 2004</td>
<td>Cross-sectional study part of ISAAC project, assessing prevalence of asthma and allergy in school children</td>
<td>Germany, European</td>
<td>Children</td>
<td>3005</td>
<td><strong>Asthma</strong> parent-reported DDA (Criteria: N/A)</td>
</tr>
</tbody>
</table>

Family studies (n = 3)

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Study population</th>
<th>Location, ethnicity</th>
<th>Adults/children</th>
<th>Sample size*</th>
<th>Definition of disease outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>David 2003</td>
<td>Family study based on case–parent triad design and focused on gene–environment interactions</td>
<td>Mexico, Latin American</td>
<td>Children</td>
<td>218 families</td>
<td><strong>Asthma</strong> diagnosed by a physician (Criteria: included skin-prick and pulmonary function testing)</td>
</tr>
<tr>
<td>Brasch-Andersen 2004</td>
<td>Family study that recruited atopic families with asthmatic children in two separate samples. Sample A: asthma; Sample B: atopic asthma</td>
<td>Denmark, European</td>
<td>Children</td>
<td>246 families</td>
<td>(Sample A) <strong>Asthma</strong> diagnosed by a physician (Criteria: clinical symptoms plus positive methacholine challenge)</td>
</tr>
<tr>
<td>Southampton</td>
<td>UK multicentre family study that recruited families with two siblings diagnosed with asthma</td>
<td>UK, European</td>
<td>Children and young adults</td>
<td>341 families</td>
<td><strong>Asthma</strong> self and parent reported (Criteria: three positive responses to: ‘Have you ever had asthma?’ and, ‘Was this confirmed by a doctor?’ and, ‘Have you used any medicines to treat asthma, or any breathing problems, at any time in the last 12 months?’)</td>
</tr>
</tbody>
</table>

N/A: not available; DDA: doctor-diagnosed asthma; GINA: global initiative on asthma.

*When results are reported for more genotypes or outcomes, the largest sample size is reported.

ECRHS: European Community Respiratory Health Survey; SPT: Skin prick tests; TOT: total; SAPALDIA: Swiss Cohort Study on Air Pollution and Lung and Heart Diseases in Adults; CCAAPS: Cincinnati Childhood Allergy and Air Pollution Study; ISAAC: International Study of Asthma and Allergies in Childhood
<table>
<thead>
<tr>
<th>Study, year</th>
<th>Affected</th>
<th>Non-affected</th>
<th>Affected</th>
<th>Non-affected</th>
<th>GSTP1 (Ile105Ile/Ile105Val/Val105Val)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fryer, 2000⁹</td>
<td>72/53</td>
<td>24/20</td>
<td>24/103</td>
<td>7/37</td>
<td>54/66/7 10/26/8 -0.046 (0.36)</td>
</tr>
<tr>
<td>Chung, 2002²⁹</td>
<td>16/17</td>
<td>31/35</td>
<td>21/12</td>
<td>34/32</td>
<td>25/8/0 51/15/0 -0.013 (0.59)</td>
</tr>
<tr>
<td>Friedin, 2002⁶⁰</td>
<td>52/17</td>
<td>42/15</td>
<td>24/45</td>
<td>15/42</td>
<td></td>
</tr>
<tr>
<td>Sideleva, 2002⁶¹</td>
<td>83/26</td>
<td>43/47</td>
<td>73/36</td>
<td>21/69</td>
<td></td>
</tr>
<tr>
<td>Vavilin, 2002³⁹</td>
<td>52/48</td>
<td>44/60</td>
<td>26/74</td>
<td>12/92</td>
<td>54/77/10 32/61/3 -0.091 (&lt;0.01)</td>
</tr>
<tr>
<td>Safronova, 2003³⁸</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aynacioglu, 2004⁴¹</td>
<td>148/120</td>
<td>1394/1343</td>
<td>42/226</td>
<td>477/2258</td>
<td>109/93/8 134/99/32 0.026 (0.04)</td>
</tr>
<tr>
<td>Kabesch, 2004⁵³</td>
<td>42/43</td>
<td>20/65</td>
<td>55/30</td>
<td>35/50</td>
<td></td>
</tr>
<tr>
<td>Saadat, 2004⁴</td>
<td>64/37</td>
<td>42/61</td>
<td>27/74</td>
<td>25/78</td>
<td>33/45/23 49/46/8 -0.013 (0.64)</td>
</tr>
<tr>
<td>Tamer, 2004¹¹</td>
<td>49/11</td>
<td>18/42</td>
<td>43/17</td>
<td>7/53</td>
<td></td>
</tr>
<tr>
<td>Zhang, 2004³⁶</td>
<td>49/33</td>
<td>97/87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee, 2005¹⁰</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>62/18/2 112/64/8 -0.004 (1.00)</td>
</tr>
<tr>
<td>Nickel, 2005¹³</td>
<td>38/39/6</td>
<td>54/60/8</td>
<td>30/36/5</td>
<td>0.000 (1.00)</td>
<td></td>
</tr>
<tr>
<td>Oh, 2005³⁷</td>
<td></td>
<td></td>
<td>102/45/7</td>
<td>75/39/5</td>
<td></td>
</tr>
<tr>
<td>Arbag, 2006⁶⁵</td>
<td>12/19</td>
<td>47/55</td>
<td>11/20</td>
<td>24/78</td>
<td>174/116/22 128/98/25 0.013 (0.36)</td>
</tr>
<tr>
<td>Ercan, 2006¹</td>
<td>124/179</td>
<td>100/151</td>
<td>64/246</td>
<td>48/202</td>
<td>137/123/26 94/95/21 -0.006 (0.75)</td>
</tr>
<tr>
<td>Holla, 2006⁶⁶</td>
<td>166/140</td>
<td>166/165</td>
<td>59/247</td>
<td>73/258</td>
<td>32/4/14 9/2/29 0.163 (&lt;0.01)</td>
</tr>
<tr>
<td>Plutecka, 2006³³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdel-Alim, 2007⁸⁰</td>
<td>84/37</td>
<td>111/115</td>
<td>41/80</td>
<td>67/159</td>
<td>63/45/13 78/107/41 0.007 (0.68)</td>
</tr>
<tr>
<td>Hanene, 2007⁵</td>
<td>73/71</td>
<td>2249/2029</td>
<td>25/119</td>
<td>797/3481</td>
<td></td>
</tr>
<tr>
<td>Imboden, 2007⁷¹</td>
<td>178/189</td>
<td>326/287</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kamada, 2007a⁶⁹</td>
<td>57/57</td>
<td>95/80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kamada, 2007b⁶⁹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mak, 2007⁸</td>
<td>161/150</td>
<td>185/130</td>
<td>144/167</td>
<td>168/147</td>
<td>207/94/11 214/91/9 -0.001 (1.00)</td>
</tr>
<tr>
<td>Salam, 2007⁷</td>
<td>240/215</td>
<td>1226/1300</td>
<td>91/563</td>
<td>537/1992</td>
<td>171/230/65 1043/1229/343 -0.004 (0.37)</td>
</tr>
<tr>
<td>ALSPAC children</td>
<td>419/333</td>
<td>2419/2156</td>
<td>124/621</td>
<td>786/3769</td>
<td>307/360/92 1985/2066/585 0.005 (0.10)</td>
</tr>
<tr>
<td>[419/249/49]</td>
<td>[2419/1689/306]</td>
<td>[124/313/227]</td>
<td>[786/1917/1276]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALSPAC mothers</td>
<td>239/223</td>
<td>3523/3064</td>
<td>74/386</td>
<td>1069/5488</td>
<td>337/377/105 2684/2935/755 -0.003 (0.26)</td>
</tr>
<tr>
<td>[239/179/28]</td>
<td>[3523/2388/435]</td>
<td>[74/164/148]</td>
<td>[1069/2787/1782]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Continued)
The study by Brasch-Andersen and colleagues, consisting of 246 Danish atopic families (452 asthmatic children plus their parents) recruited in two separate samples, evaluated the risk of asthma and atopic asthma associated with the three genes. GSTM1 and GSTT1 were analysed as CNV with full gene-dosage information available. The study found strong evidence of an association of the GSTM1 homozygous deletion with asthma ($P < 0.0005$), which became even stronger when limiting the analyses to atopic asthma ($P < 0.00005$). An association of GSTT1 with asthma was also found, but under an additive model ($P = 0.019$), with similar results obtained for atopic asthma ($P = 0.021$), whereas no association was found with GSTP1.

The study by David and colleagues, consisting of 218 Mexican case–parent triads, focused on the interaction between NQO1 (Pro187Ser polymorphism) and GSTM1 genes in increasing the risk of asthma in children exposed to high level of ozone (Mexico City). The study, which evaluated GSTM1 in terms of null genotype (homozygous deletion), did not assess the effect of GSTM1 alone, but showed a protective effect of the NQO1 Ser allele in children with null GSTM1 genotype.

### Quality of the studies included

We assessed the quality of the 27 articles reporting on the 25 studies included in the meta-analyses, and describe the study populations and methods used for the meta-analyses. All studies described the genotyping methods used. Genotyping error was evaluated in a quarter of them, mainly by repeating the genotyping in 5–15% of the samples, and no errors were found in most cases. However, only 14% of the studies reported on the potential problem of population stratification, although only a few discussed the potential problem in detail. Four studies were identified as being of poor quality, including three case–control studies with small sample size (less than 500), and one cohort study. Only two of them (33) reported findings of null genotype alone. All studies described the genotyping methods used.

### Table 2

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Affected</th>
<th>Non-affected</th>
<th>Affected</th>
<th>Non-affected</th>
<th>Disequilibrium Coefficient ($P$-value)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMICS-INMA</td>
<td>11/7</td>
<td>224/169</td>
<td>4/14</td>
<td>74/319</td>
<td>-0.016 (0.17)</td>
</tr>
<tr>
<td>Wheezing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilliland, 2002</td>
<td>455/529</td>
<td>816/1010</td>
<td>114/530</td>
<td>898/4415</td>
<td>0.016 (0.17)</td>
</tr>
<tr>
<td>Lee, 2008</td>
<td>128/85</td>
<td>99/85</td>
<td>7/29</td>
<td>71/297</td>
<td>0.046 (0.36)</td>
</tr>
<tr>
<td>Schroer, 2009</td>
<td>358/289</td>
<td>2831/2513</td>
<td>210/251/64</td>
<td>1206/1321/365</td>
<td>0.001 (0.85)</td>
</tr>
<tr>
<td>ALSPAC children</td>
<td>327/298</td>
<td>1995/1802</td>
<td>115/510</td>
<td>253/266/48</td>
<td>0.004 (0.17)</td>
</tr>
<tr>
<td>AMICS-INMA study</td>
<td>278/246</td>
<td>1515/1391</td>
<td>81/437</td>
<td>477/2407</td>
<td>0.000 (0.008)</td>
</tr>
</tbody>
</table>

$^a$HWE tested (exact test) among controls in case–control studies, and in the whole population otherwise.

The study by Brasch-Andersen and colleagues, consisting of 246 Danish atopic families (452 asthmatic children), was the only study in the meta-analysis to evaluate the interaction between NQO1 and GSTM1 genes, and found an association of the GSTM1 homozygous deletion with asthma ($P = 0.0019$), with similar results obtained for atopic asthma ($P < 0.0005$). An association of GSTT1 with asthma was also found, but under an additive model ($P = 0.019$), whereas no association was found with GSTP1.

The study by David and colleagues, consisting of 218 Mexican case–parent triads, focused on the interaction between NQO1 (Pro187Ser polymorphism) and GSTM1 genes in increasing the risk of asthma in children exposed to high level of ozone (Mexico City). The study, which evaluated GSTM1 in terms of null genotype (homozygous deletion), did not assess the effect of GSTM1 alone, but showed a protective effect of the NQO1 Ser allele in children with null GSTM1 genotype.
mentioned the possibility that population stratification might have occurred.

**Meta-analyses on GSTM1 deletion polymorphism**

A total of 19 published and 3 primary studies evaluated the association of GSTM1 and asthma, including a total of 4416 affected and 23902 non-affected individuals. The meta-analysis in Figure 2a shows an increased risk of asthma associated with the GSTM1 null genotype (pooled OR 1.28; 95% CI 1.09–1.52). However, large between-study heterogeneity was observed ($I^2 = 76\%$), which could not be explained by age (adults/children) or ethnicity, as approximated by study geographical location (continent). These subgroup analyses, however, showed that most heterogeneity was present in studies on adults and in those performed in Asia (Supplementary Figure S2 available as supplementary data at IJE online). Similarly, exclusion of two studies with poor quality$^{36,39}$ reduced the pooled OR to 1.19 (1.03–1.38) with little reduction of the $I^2$ (69%). The subgroup analysis by asthma definition (Figure 2b) could not explain heterogeneity either, although it showed that heterogeneity was limited to studies where asthma was diagnosed by a physician and where asthma was not defined in the article, whereas it was absent among studies with self-reported asthma. However, studies using self-reported asthma included those with the largest sample size, therefore it is difficult to disentangle the impact of asthma definition from that of study size on the observed heterogeneity. In fact, the funnel plot in Figure 2c strongly supports the presence of small-study bias. When the meta-analysis was repeated by limiting inclusion to the nine studies at the top of the funnel, the heterogeneity dropped from 76 to 38% and the OR became very close to 1 (1.02; 0.92–1.13).

As for the association of GSTM1 null genotype with wheezing and BHR, only four and three studies were available, respectively. As shown in Figure 5a, for both outcomes results were very homogeneous across studies. The meta-analysis showed no effect on BHR (1.01; 0.90–1.15), and only a possible small effect on wheezing (1.08; 0.99–1.19).

**Meta-analyses on GSTT1 deletion polymorphism**

A total of 16 published and 3 primary studies evaluated the association of GSTT1 and asthma, including 3852 affected and 22880 non-affected individuals. Similarly to GSTM1, the meta-analysis in Figure 3a shows an OR of 1.39 (1.09–1.77), but with extreme between-study heterogeneity ($I^2 = 81\%$). As for GSTM1, heterogeneity was absent within the subgroup of studies with self-reported asthma (Figure 3b), and small-study bias was the only factor substantially explaining heterogeneity. When restricting the meta-analysis to the nine largest studies at the top of the funnel plot in Figure 3c, the OR became 0.93 (0.84–1.03) and the heterogeneity disappeared ($I^2 = 0\%$).

On the contrary, exclusion of the two studies with poor quality$^{36,39}$ reduced the pooled OR to 1.19 (0.97–1.44), but did not reduce substantially the degree of heterogeneity ($I^2 = 70\%$). Subgroup analyses by age and ethnicity did not provide additional insight on the causes of heterogeneity (Supplementary Figure S3 available as supplementary data at IJE online).

Similarly to GSTM1, the association of GSTT1 with wheezing and BHR (Figure 5b) showed homogeneous results across studies (two and three studies included, respectively). The meta-analyses showed no genetic effect for either outcome (wheezing 1.05; 0.86–1.30; BHR 0.96; 0.81–1.13).

**Meta-analyses on GSTP1 Ile105Val polymorphism**

A total of 14 published and 3 primary studies with 3363 affected and 14442 non-affected individuals were available for the meta-analysis of GSTP1 Ile/Val vs Ile/Ile and asthma, whereas 13 published and 3 primary studies with 2160 affected and 9034 non-affected individuals were available for the contrast Val/Val vs Ile/Ile (Figure 4; Supplementary Figure S4 and S5 available as supplementary data at IJE online). The meta-analyses in Figure 4 show no clear association of GSTP1 with asthma, although the two pooled estimates point to a possible protective effect of the Val allele, with an OR of 0.93 (0.82–1.06) for Ile/Val vs Ile/Ile and 0.79 (0.57–1.08) for Val/Val vs Ile/Ile. Although between-study heterogeneity was moderate for Ile/Val vs Ile/Ile ($I^2 = 39\%$), it was large for Val/Val vs Ile/Ile (72%), so that the result for the latter is difficult to interpret. Heterogeneity could not be explained by small-study bias (Supplementary Figure S5c available as supplementary data at IJE online), and was not reduced by removing the two studies with poor quality$^{35,37}$ either. Ethnicity did explain some heterogeneity (Supplementary Figure S5b available as supplementary data at IJE online), and exclusion of the two North African studies (Egypt and Tunisia) brought the ORs close to 1 (Ile/Val vs Ile/Ile 0.98; 0.87–1.10, and Val/Val vs Ile/Ile 0.94; 0.71–1.24) and reduced $I^2$ to 59%. Pooling of the two African studies showed a marked protective effect (Ile/Val vs Ile/Ile 0.52; 0.33–0.83, and Val/Val vs Ile/Ile 0.24; 0.09–0.69). The subgroup analysis by asthma definition showed similar results as for GSTM1 and GSTT1, with no heterogeneity within studies with self-reported asthma (Figure 4c and d).

Only three studies were available for the association of GSTP1 with wheezing and BHR, and meta-analysis could only be performed for the comparison of Ile/Val vs Ile/Ile where the heterogeneity was small (Figure 5c and d). For the Ile/Val vs Ile/Ile comparison there is a suggestion of an increased risk associated with the Val allele for both outcomes. Effects in opposite directions are shown for the Val/Val vs Ile/Ile comparison, with the Val allele having a protective effect on BHR as with asthma, but being associated
with increased risk of wheezing. These findings need to be interpreted with caution since they are driven by two relatively small studies, the study by Fryer\(^\text{9}\) and the AMICS-INMA study, respectively.

The results of the analyses based on the model-free approach were similar to those based on between-genotype comparisons, giving an OR of 0.93 (0.77–1.03) for Ile/Val vs Ile/Ile and 0.77 (0.49–1.14) for Val/Val vs Ile/Ile. The model-free approach provided an estimate of \(\lambda\), the parameter indicating the underlying genetic model, of 0.27 (0.01–0.61), where 0 corresponds to the recessive, 0.5 to the co-dominant and 1 to the dominant model.\(^\text{26}\) Despite the wide CI, the estimate of \(\lambda\) seems to exclude a dominant genetic model and suggests either a co-dominant or a recessive model.

**Strength of the evidence from the meta-analyses**

When applying the Venice criteria to assess credibility,\(^\text{25}\) the cumulative evidence reviewed does not clearly support a genetic effect for any of the three \(\text{GST}\) genes on asthma. In fact, although the amount of evidence is good for all three meta-analyses, particularly those on \(\text{GSTMI}\) and \(\text{GSTTI}\), the degree of heterogeneity across studies and the likely presence of publication bias make the credibility of the evidence weak. Moreover, when limiting the meta-analyses to the largest studies, positive findings shown by other studies could not be replicated, suggesting that a genetic effect is likely to be either absent or very small.

**Discussion**

Overall, the evidence synthesized in this review does not support a substantial role of \(\text{GST}\) genes on asthma phenotypes in either children or adults, although small effects cannot be excluded and it is possible that these genes act on airway disease through interaction with environmental exposures or other genes. The weakness of the available evidence in supporting an effect of \(\text{GST}\) genes on asthma is mainly due to publication bias and large heterogeneity of study results. Interestingly, the results from the three

\[
\begin{array}{|l|l|l|l|}
\hline
\text{Study} & \text{Location} & \text{OR (95\% CI)} & \% \text{Weight} \\
\hline
\text{Fryer, 2000} & \text{UK} & 1.13 (0.57, 2.26) & 3.24 \\
\text{Chung, 2002} & \text{Korea} & 1.06 (0.46, 2.45) & 2.57 \\
\text{Freidin, 2002} & \text{Asian Russia} & 1.09 (0.49, 2.44) & 2.70 \\
\text{Sideleva, 2002} & \text{European Russia} & 3.49 (1.91, 6.38) & 3.73 \\
\text{Vavilin, 2002} & \text{European Russia} & 1.48 (0.85, 2.57) & 4.05 \\
\text{Kabesch, 2004} & \text{Germany} & 1.19 (0.92, 1.53) & 6.27 \\
\text{Saadat, 2004} & \text{Iran} & 3.17 (1.65, 6.12) & 3.42 \\
\text{Tamer, 2004} & \text{Asian Turkey} & 2.51 (1.43, 4.42) & 3.98 \\
\text{Zhang, 2004} & \text{China} & 10.39 (4.42, 24.46) & 2.50 \\
\text{Lee, 2005} & \text{Taiwan} & 1.33 (0.79, 2.26) & 4.22 \\
\text{Arbag, 2006} & \text{Asian Turkey} & 0.74 (0.33, 1.68) & 2.63 \\
\text{Ercan, 2006} & \text{Asian Turkey} & 1.05 (0.74, 1.47) & 5.60 \\
\text{Holla, 2006} & \text{Czech Republic} & 1.18 (0.86, 1.61) & 5.83 \\
\text{Hanene, 2007} & \text{Tunisia} & 2.35 (1.48, 3.75) & 4.65 \\
\text{Imboden, 2007} & \text{Switzerland} & 0.93 (0.67, 1.29) & 5.67 \\
\text{Kamada_1, 2007} & \text{Japan} & 0.83 (0.64, 1.07) & 6.22 \\
\text{Kamada_2, 2007} & \text{Japan} & 0.84 (0.53, 1.35) & 4.61 \\
\text{Mak, 2007} & \text{China} & 0.75 (0.55, 1.03) & 5.80 \\
\text{Salam, 2007} & \text{USA} & 1.18 (0.97, 1.46) & 6.82 \\
\text{ALSPAC children} & \text{UK} & 1.12 (0.96, 1.31) & 6.89 \\
\text{ALSPAC mothers} & \text{UK} & 0.93 (0.77, 1.13) & 6.69 \\
\text{AMICS-INMA} & \text{Spain} & 1.19 (0.45, 3.12) & 2.11 \\
\hline
\text{Overall (} \chi^2 = 76.0\%, \ P = 4.4e-10) & & 1.28 (1.09, 1.52) & 100.00 \\
\hline
\end{array}
\]

**Figure 2** Random effects meta-analysis of \(\text{GSTMI}\) (null vs present) effect on asthma: (a) forest plot for the main analysis; (b) forest plot for the subgroup analysis by asthma diagnosis; (c) funnel plot, where studies classified as ‘small’ or ‘large’ in the sensitivity analyses are represented by dots and triangles, respectively (Egger’s test: \(P = 0.035\); Begg’s test: \(P = 0.135\))
(b) Table 1. Study characteristics and outcomes for asthma and self/parent-reported asthma.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma diagnosed by a physician</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fryer, 2000</td>
<td>UK</td>
<td>1.13 (0.57, 2.26)</td>
</tr>
<tr>
<td>Chung, 2002</td>
<td>Korea</td>
<td>1.06 (0.46, 2.45)</td>
</tr>
<tr>
<td>Freidin, 2002</td>
<td>Asian Russia</td>
<td>1.09 (0.49, 2.44)</td>
</tr>
<tr>
<td>Sideleva, 2002</td>
<td>European Russia</td>
<td>3.49 (1.91, 6.38)</td>
</tr>
<tr>
<td>Saadat, 2004</td>
<td>Iran</td>
<td>3.17 (1.65, 6.12)</td>
</tr>
<tr>
<td>Tamer, 2004</td>
<td>Asian Turkey</td>
<td>2.51 (1.43, 4.42)</td>
</tr>
<tr>
<td>Arbag, 2006</td>
<td>Asian Turkey</td>
<td>0.74 (0.33, 1.68)</td>
</tr>
<tr>
<td>Ercan, 2006</td>
<td>Asian Turkey</td>
<td>1.05 (0.74, 1.47)</td>
</tr>
<tr>
<td>Holla, 2006</td>
<td>Czech Republic</td>
<td>1.18 (0.86, 1.61)</td>
</tr>
<tr>
<td>Kamada_1, 2007</td>
<td>Japan</td>
<td>0.83 (0.64, 1.07)</td>
</tr>
<tr>
<td>Kamada_2, 2007</td>
<td>Japan</td>
<td>0.84 (0.53, 1.35)</td>
</tr>
<tr>
<td>Mak, 2007</td>
<td>China</td>
<td>0.75 (0.55, 1.03)</td>
</tr>
<tr>
<td><strong>Subtotal (I² = 75.3%, P = 5.7e-06)</strong></td>
<td>1.24 (0.94, 1.63)</td>
<td></td>
</tr>
<tr>
<td><strong>Self/parent-reported asthma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kabeschi, 2004</td>
<td>Germany</td>
<td>1.19 (0.92, 1.53)</td>
</tr>
<tr>
<td>Lee, 2005</td>
<td>Taiwan</td>
<td>1.33 (0.79, 2.26)</td>
</tr>
<tr>
<td>Imboden, 2007</td>
<td>Switzerland</td>
<td>0.93 (0.67, 1.32)</td>
</tr>
<tr>
<td>Salam, 2007</td>
<td>USA</td>
<td>1.18 (0.97, 1.45)</td>
</tr>
<tr>
<td>ALSPAC children</td>
<td>UK</td>
<td>1.12 (0.96, 1.31)</td>
</tr>
<tr>
<td>ALSPAC mothers</td>
<td>UK</td>
<td>0.93 (0.77, 1.13)</td>
</tr>
<tr>
<td>AMICS-INMA</td>
<td>Spain</td>
<td>1.19 (0.45, 3.12)</td>
</tr>
<tr>
<td><strong>Subtotal (I² = 0.0%, P = 0.499)</strong></td>
<td>1.09 (0.99, 1.19)</td>
<td></td>
</tr>
<tr>
<td><strong>Asthma not defined</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vavilin, 2002</td>
<td>European Russia</td>
<td>1.48 (0.85, 2.57)</td>
</tr>
<tr>
<td>Hanene, 2007</td>
<td>Tunisia</td>
<td>2.35 (1.48, 3.75)</td>
</tr>
<tr>
<td><strong>Subtotal (I² = 85.9%, P = 0.001)</strong></td>
<td>3.11 (1.23, 7.85)</td>
<td></td>
</tr>
<tr>
<td>Overall (I² = 76.0%, P = 4.4e-10)</td>
<td>1.28 (1.09, 1.52)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis.

(c) Figure 2 Continued
unpublished studies on GST genes and asthma phenotypes were negative. The only exception was the association between GSTP1 and wheezing (but not asthma) in the AMICS-INMA study, which suggested an increased risk for the Val allele. It is important to note that the ALSPAC, which provided negative results consistently for all GST genes and asthma phenotypes, was the largest study included in all the meta-analyses. In fact, the plan for the systematic review and meta-analysis presented here originated with the aim of putting the results of the ALSPAC cohort into context, and understand the reasons for the apparent disagreement with previous published studies. The lack of evidence of an important role of GST genes in the development of asthma, wheezing and BHR is in agreement with negative findings on lung function in children for all three GST genes from the ALSPAC (data not shown), although an association between GSTM1 and GSTP1 genes and lung function in childhood has been previously suggested.43

Although the meta-analyses on GSTM1 and GSTT1 with inclusion of all studies showed an increased risk of asthma associated with the two null genotypes, the presence of small-study bias and extreme heterogeneity in study results make the credibility of these findings very low. Despite our efforts to be as inclusive as possible (comprehensive search strategy, avoidance of language restrictions, inclusion of conference abstracts), the meta-analyses showed clear absence of small studies with negative results, suggesting the presence of publication bias. This is an important issue in meta-analysis of genetic association studies, which needs to be highlighted and addressed. We did so by repeating the analyses with inclusion of only the largest studies, and we found loss of the observed associations, with ORs very close to 1. Positive results were not only confined to small studies, but also to studies with poor quality, the exclusion of which caused similar loss of the observed associations. In support of an absence of an association of GSTM1 and GSTT1 with airway disease are the negative findings of the meta-analyses on wheezing and BHR. An exception might be the association between GSTM1 and wheezing, where the

![Figure 3](https://via.placeholder.com/150)

**Figure 3** Random effects meta-analysis of GSTT1 (null vs present) effect on asthma: (a) forest plot for the main analysis; (b) forest plot for the subgroup analysis by asthma diagnosis; (c) funnel plot, where studies classified as ‘small’ or ‘large’ in the sensitivity analyses are represented by dots and triangles, respectively (Egger's test: $P = 0.003$; Begg's test: $P = 0.001$)
meta-analysis suggests a possible small effect of the null genotype in increasing the risk of \( \sim 10\%\). Although the studies included in the meta-analyses on wheezing and BHR were only few and the results supported only weak evidence of associations, the consistency in results and narrow CIs of the ORs give some credibility to these findings. An important methodological point regarding studies on \textit{GSTT1} or...
**Figure 4** Random effects meta-analysis of GSTP1 effect on asthma: (a) Ile/Val vs Ile/Ile; (b) Val/Val vs Ile/Ile; (c) subgroup analysis by asthma diagnosis for Ile/Val vs Ile/Ile; (d) subgroup analysis by asthma diagnosis for Val/Val vs Ile/Ile

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fryer, 2000</td>
<td>UK</td>
<td>0.47 (0.21, 1.08)</td>
<td>2.19</td>
</tr>
<tr>
<td>Chung, 2002</td>
<td>Korea</td>
<td>1.09 (0.41, 2.91)</td>
<td>1.56</td>
</tr>
<tr>
<td>Safronova, 2003</td>
<td>European Russia</td>
<td>0.75 (0.43, 1.30)</td>
<td>4.19</td>
</tr>
<tr>
<td>Aynacioglu, 2004</td>
<td>Asian Turkey</td>
<td>1.15 (0.79, 1.69)</td>
<td>7.08</td>
</tr>
<tr>
<td>Tamer, 2004</td>
<td>Asian Turkey</td>
<td>1.45 (0.79, 2.65)</td>
<td>3.63</td>
</tr>
<tr>
<td>Lee, 2005</td>
<td>Taiwan</td>
<td>0.51 (0.28, 0.93)</td>
<td>3.59</td>
</tr>
<tr>
<td>Nickel, 2005</td>
<td>Germany</td>
<td>0.92 (0.52, 1.66)</td>
<td>3.88</td>
</tr>
<tr>
<td>Oh, 2005</td>
<td>Korea</td>
<td>0.85 (0.50, 1.43)</td>
<td>4.55</td>
</tr>
<tr>
<td>Ercan, 2006</td>
<td>Asian Turkey</td>
<td>0.87 (0.61, 1.24)</td>
<td>7.73</td>
</tr>
<tr>
<td>Plutecka, 2006</td>
<td>Poland</td>
<td>0.89 (0.61, 1.29)</td>
<td>7.18</td>
</tr>
<tr>
<td>Abdel-Alim, 2007</td>
<td>Egypt</td>
<td>0.56 (0.09, 3.58)</td>
<td>0.47</td>
</tr>
<tr>
<td>Hanene, 2007</td>
<td>Tunisia</td>
<td>0.52 (0.32, 0.84)</td>
<td>5.14</td>
</tr>
<tr>
<td>Mak, 2007</td>
<td>China</td>
<td>1.07 (0.76, 1.51)</td>
<td>7.91</td>
</tr>
<tr>
<td>Salam, 2007</td>
<td>USA</td>
<td>1.14 (0.92, 1.41)</td>
<td>12.26</td>
</tr>
<tr>
<td>ALSPAC children</td>
<td>UK</td>
<td>1.16 (0.98, 1.37)</td>
<td>14.27</td>
</tr>
<tr>
<td>ALSPAC mothers</td>
<td>UK</td>
<td>0.88 (0.72, 1.07)</td>
<td>12.88</td>
</tr>
<tr>
<td>AMICS-INMA</td>
<td>Spain</td>
<td>0.97 (0.36, 2.63)</td>
<td>1.50</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>0.93 (0.82, 1.06)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

NOTE: Weights are from random effects analysis
NOTE: Weights are from random effects analysis.

Overall (I² = 39.3%, P = 0.049)

Asthma not defined
Hanene, 2007
Oh, 2005
Tamer, 2004
Nickel, 2005
Subtotal (I² = 0.0%, P = 0.479)
Lee, 2005
Plutecka, 2006
Safronova, 2003
ALSPAC children
Aynacioglu, 2004
Subtotal (I² = 6.4%, P = 0.361)
ALSPAC mothers
Lee, 2005
Plutecka, 2006
Safronova, 2003
AMICS-INMA
Asthma diagnosed by a physician
Fryer, 2000
Chung, 2002
Aynacioglu, 2004
Tamer, 2004
Nickel, 2005
Ercan, 2006
Abed-Alim, 2007
Mak, 2007
Subtotal (I² = 62.4%, P = 0.031)
Study
Fryer, 2000
Aynacioglu, 2004
Tamer, 2004
Nickel, 2005
Ercan, 2006
Abed-Alim, 2007
Mak, 2007
Location
UK
Korea
Asian Turkey
Asian Turkey
Germany
Asian Turkey
Egypt
China
OR (95% CI)
0.93 (0.82, 1.06)
1.45 (0.79, 2.65)
0.92 (0.52, 1.65)
0.87 (0.61, 1.24)
0.56 (0.09, 3.58)
0.90 (0.47, 1.73)
0.51 (0.28, 0.93)
0.77 (0.43, 1.37)
Overall (I² = 45.9%, P = 0.136)
Study
Fryer, 2000
Aynacioglu, 2004
Tamer, 2004
Nickel, 2005
Ercan, 2006
Abed-Alim, 2007
Mak, 2007
Location
UK
Asian Turkey
Asian Turkey
German
Asian Turkey
Egypt
China
OR (95% CI)
1.98 (0.51, 7.71)
1.03 (0.31, 3.37)
0.85 (0.45, 1.60)
0.39 (0.19, 0.80)
0.78 (0.42, 1.43)
Overall (I² = 72.1%, P = 2.8e-06)
Study
Fryer, 2000
Aynacioglu, 2004
Tamer, 2004
Nickel, 2005
Ercan, 2006
Abed-Alim, 2007
Mak, 2007
Location
UK
Asian Turkey
Asian Turkey
German
Asian Turkey
Egypt
China
OR (95% CI)
0.79 (0.57, 1.06)

Figure 4  Continued

(c)

Study     Location     OR (95% CI)

Asthma diagnosed by a physician
Fryer, 2000      UK    0.47 (0.21, 1.06)
Chung, 2002     Korea  1.09 (0.41, 2.91)
Aynacioglu, 2004 Asian Turkey  1.15 (0.79, 1.69)
Tamer, 2004     Asian Turkey  1.45 (0.79, 2.65)
Nickel, 2005    Germany  0.92 (0.52, 1.65)
Ercan, 2006     Asian Turkey  0.87 (0.61, 1.24)
Abed-Alim, 2007 Egypt  0.56 (0.09, 3.58)
Mak, 2007       China   1.07 (0.76, 1.51)
Subtotal      (I² = 0.0%, P = 0.479)

Self/parent-reported asthma
Lee, 2005       Taiwan  0.51 (0.28, 0.93)
Salam, 2007     USA     1.14 (0.92, 1.41)
ALSPAC children UK      1.16 (0.98, 1.37)
ALSPAC mothers UK      0.88 (0.72, 1.07)
AMICS-INMA     Spain    0.97 (0.36, 2.63)
Subtotal      (I² = 62.4%, P = 0.031)

Asthma not defined
Safronova, 2003 European Russia  0.75 (0.43, 1.30)
Oh, 2005        Korea    0.85 (0.50, 1.43)
Plutecka, 2006  Poland   0.89 (0.61, 1.29)
Hanene, 2007    Tunisia  0.52 (0.32, 0.84)
Subtotal      (I² = 6.4%, P = 0.361)

Overall (I² = 39.3%, P = 0.049)

Note: Weights are from random effects analysis.

(d)

Study     Location     OR (95% CI)

Asthma diagnosed by a physician
Fryer, 2000      UK    0.16 (0.05, 0.55)
Aynacioglu, 2004 Asian Turkey  0.31 (0.14, 0.69)
Tamer, 2004     Asian Turkey  4.27 (1.71, 10.69)
Nickel, 2005    Germany  1.07 (0.34, 3.32)
Ercan, 2006     Asian Turkey  0.65 (0.35, 1.20)
Abed-Alim, 2007 Egypt  2.14 (0.05, 0.36)
Mak, 2007       China    1.26 (0.51, 3.11)
Subtotal      (I² = 83.8%, P = 1.7e-06)

Self/parent-reported asthma
Lee, 2005       Taiwan  0.45 (0.09, 2.19)
Salam, 2007     USA     1.16 (0.85, 1.58)
ALSPAC children UK      1.02 (0.79, 1.31)
ALSPAC mothers UK      1.09 (0.81, 1.45)
AMICS-INMA     Spain    1.34 (0.97, 2.77)
Subtotal      (I² = 6.4%, P = 0.808)

Asthma not defined
Safronova, 2003 European Russia  1.98 (0.51, 7.71)
Oh, 2005        Korea    1.03 (0.31, 3.37)
Plutecka, 2006  Poland   0.85 (0.45, 1.60)
Hanene, 2007    Tunisia  0.39 (0.19, 0.80)
Subtotal      (I² = 45.9%, P = 0.136)

Overall (I² = 72.1%, P = 2.8e-06)

Note: Weights are from random effects analysis.
*GSTM1* is that methods currently used to evaluate these genes cannot distinguish between genotypes with one or two copies of the gene. By classifying the genotype as ‘present’ or ‘null’ they imply a recessive model (one or two copies vs absence of the risk allele), which may not reflect the true underlying genetic model and thus may not provide a valid and accurate estimate of the genetic risk. *GSTT1* or *GSTM1* CNVs (also known as ‘gene dosage’) are correlated with altered enzyme activity, and analysis in a dose-dependent manner would best describe any disease outcome association. Brasch–Andersen and colleagues have shown that it is possible to utilize the increased sensitivity of real-time PCR assays to provide dosage of *GSTT1* and *GSTM1*. A similar approach

**Figure 5** Results on wheezing and BHR for: (a) *GSTM1*; (b) *GSTT1*; (c) *GSTP1* Ile/Val vs Ile/Ile; (d) *GSTP1* Val/Val vs Ile/Ile. Fixed effects meta-analysis was performed for *GSTM1*, *GSTT1* and *GSTP1* Ile/Val vs Ile/Ile.
was utilized to obtain gene-dosage information in the ALSPAC cohort.\textsuperscript{17}

The meta-analysis on \textit{GSTP1} Ile105Val polymorphism and asthma suggested a possible, weak protective effect of the Val allele. The Val substitution in the \textit{GSTP1} gene is associated with altered substrate affinities compared with the Ile105 wild-type allele\textsuperscript{46} and heterozygosity has also been associated with reduced risk of chronic obstructive pulmonary disease.\textsuperscript{47} Although in the meta-analysis on \textit{GSTP1} and asthma there was no clear evidence of small-study bias, heterogeneity in study results was large and limited the interpretability of the pooled estimates. Subgroup analyses showed a potential role of ethnicity in explaining such heterogeneity, with a protective effect of the Val allele limited to the two North African studies, where the association was very strong. This finding might be explained by the presence of gene–environment or gene–gene interactions in these study populations. \textit{GST} genes effects on

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Study} & \textbf{Location} & \textbf{OR (95\% CI)} & \textbf{Weight} \\
\hline
\textbf{Wheezing} & & & \\
Schroer, 2008 & USA & 1.23 (0.76, 2.00) & 10.86 \\
ALSPAC children & UK & 1.25 (1.05, 1.49) & 85.29 \\
AMICS-INMA & Spain & 2.32 (1.03, 5.23) & 3.85 \\
Subtotal (\(I^2 = 5.6\%, \ P = 0.347\)) & & 1.28 (1.09, 1.50) & 100.00 \\
\hline
\textbf{BHR} & & & \\
Fryer, 2000 & UK & 0.53 (0.21, 1.33) & 2.12 \\
Imboden, 2007 & Switzerland & 1.14 (0.95, 1.37) & 52.65 \\
ALSPAC children & UK & 1.09 (0.89, 1.33) & 45.23 \\
Subtotal (\(I^2 = 22.1\%, \ P = 0.277\)) & & 1.10 (0.96, 1.26) & 100.00 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{Study} & \textbf{Location} & \textbf{OR (95\% CI)} \\
\hline
\textbf{Wheezing} & & \\
Schroer, 2008 & USA & 1.44 (0.77, 2.71) \\
ALSPAC children & UK & 0.90 (0.68, 1.19) \\
AMICS-INMA & Spain & 5.01 (1.72, 14.58) \\
\hline
\textbf{BHR} & & \\
Fryer, 2000 & UK & 0.18 (0.04, 0.82) \\
Imboden, 2007 & Switzerland & 0.95 (0.68, 1.32) \\
ALSPAC children & UK & 1.01 (0.74, 1.36) \\
\hline
\end{tabular}
\end{table}

\textbf{Figure 5} Continued
asthma risk may be modified by exposures that produce lung oxidative injury, such as smoking, air pollution and infections. For example, one might speculate that smoking in North African countries is a major problem, and the common waterpipe smoking seems to produce even more free radicals than cigarette smoking.\(^{58}\) The value of such speculations is limited in the absence of supporting evidence, and bias associated with selective reporting or poor quality might be an alternative explanation for the positive findings of the two North African studies. Future research in these populations should collect information on factors potentially interacting with \(GST\) genes in modifying asthma risk, including oxidative exposures. Only few studies evaluated the effects of \(GSTP1\) on wheezing and BHR, and the findings were in opposite directions for the two outcomes. The Val allele was protective for BHR, in agreement with the results for asthma, but associated with increased risk of wheezing. These effects were suggested by two relatively small studies, whereas the large ALSPAC cohort did not provide convincing evidence of any association with either outcome.

Failure to account for environmental exposures in our meta-analyses might partly explain not only the heterogeneity of results across studies, but also the overall negative findings. Strong environmental effects on asthma phenotypes could mask modest genetic effects and, more importantly, gene–environment interactions could make the effects of \(GST\) genes become substantial only in the presence of oxidative exposures and not detectable at a population level.\(^{59}\) Passive smoking, ambient air pollution and endotoxin or other pathogen-associated molecules are good candidates for gene–environment interactions in asthma, particularly with genes involved in antioxidant defence, such as the \(GST\) genes.\(^{59–51}\) In our review, variation in exposure to these environmental factors across studies is likely to have happened given the diverse geographical setting of the studies included, and gene–environment interactions might partly explain the large heterogeneity observed. Moreover, there is evidence that antioxidant supplementation can modify these gene–environment interactive effects,\(^{52}\) so that the nutritional status of the study population could represent an additional source of heterogeneity. The evaluation of gene–environment interactions is problematic due to the lack of power of statistical tests for interactions and the high measurement error present in the assessment of most environmental exposures. In fact, despite the strong biological rationale, results from the literature on gene–environment interactions in asthma remain inconclusive.\(^{51}\) Only a small proportion of the studies reviewed in this article reported on gene–environment interactions, including environmental tobacco smoke (ETS), \(^{33,39,53}\) in utero ETS\(^{33,53}\) and air pollution,\(^{7,54}\) and the same environmental exposure was assessed in different ways across studies.

Researchers interested in the effects of \(GST\) genes should be encouraged to collect information on relevant environmental exposures and carefully choose the methods to measure them. Standardization of methods for environmental exposure assessment and full reporting of the interactions tested will allow the pooling of data across studies and to reach adequate power to detect interactions. The study of possible interactions between \(GST\) genes and environmental exposures on asthma is important not only for our understanding of the etiopathogenesis of this complex disease, but also for its possible implications for public health. Asthma prevalence is high and increasing over time worldwide, and \(GST\) genes polymorphisms are frequent in the population, particularly the \(GSTM1\) null genotype, with reported frequencies in different ethnic groups varying from 18 to 66%.\(^{55}\) The possibility of implementing simple measures such as antioxidant diet supplementation in subjects at risk makes this topic worthwhile for further research.

\(GSTs\) Mu-1, Theta-1 and Pi-1 have overlapping substrate specificities, so that a deficiency in one isofrom may be compensated by another. A meta-analysis of 14 studies showed that adults with a combined \(GSTM1\) and \(GSTT1\) null genotype were at a higher risk of asthma (OR 2.15, 95% CI 1.21–3.71) and suggested that smoking overloads the capacity of either \(GSTM1\) or \(GSTT1\) detoxification systems.\(^{56}\) We investigated the effect of the combined null genotype using published data from 16 studies and unpublished data from the ALSPAC and AMICS-INMA study (data not shown). Although we found an association with increased asthma risk (OR 1.39, 95% CI 1.16–1.67), the extreme between-study heterogeneity (\(I^2 = 88\%\)) and presence of publication bias greatly limit the credibility of these findings. Moreover, the results from the ALSPAC, which represented the largest sample in the meta-analysis, were negative. On the other hand, \(GST\) genes could interact with genes coding for other detoxifying enzymes induced in response to oxidative stress. Supporting this hypothesis is some evidence of interaction between \(GSTM1\) null genotype and \(NQO1\) Pro187Ser polymorphism on asthma.\(^{34}\) Future large studies evaluating \(GST\) genes in addition to other antioxidant genes are needed to provide evidence on gene–gene interactive effects on asthma.

This systematic review only focussed on asthma risk, and did not consider the possible association of \(GST\) genes with asthma severity in patients affected by the disease. A finding from the Southampton study was the association of the \(GSTT1\) null allele with an increased severity score in patients with asthma (data not shown). There is evidence suggesting that \(GST\) genes, in particular \(GSTM1\) and \(GSTP1\), might also interact with air pollution and tobacco smoke exposures in exacerbating respiratory symptoms and decreasing lung function in asthmatic individuals.\(^{12,32,57}\)
Despite our attempt to investigate causes of heterogeneity in study results in the meta-analyses presented, we were limited by the availability of relevant information in the published reports. For example, although we did not find evidence of effect modification by ethnicity and age at onset, we cannot exclude the contribution of these factors to the observed heterogeneity, given that we used crude proxies for them (study geographical location for ethnicity, and age of study population instead of age at onset for childhood vs adult asthma). Differences in asthma definition may also have played a role in generating the observed heterogeneity. Asthma diagnosed by a physician, self-reported doctor-diagnosed asthma and self-reported history of asthma differ in sensitivity and specificity. Moreover, asthmatic individuals identified through questionnaire in a population-based study may have lower severity than patients recruited at a clinic. Although asthma definition could not explain heterogeneity in our meta-analyses, heterogeneity was not present within the subgroup of studies that used self-reported asthma, where effects were closer to the null hypothesis compared with studies that used physician-diagnosed asthma and those that did not report how asthma was defined. However, interpretation of this finding is difficult, since we could not disentangle the possible effect of asthma definition from that of study design and study size. Physician-diagnosed asthma was used by relatively small case-control studies, whereas self-reported asthma was used by large cohort and cross-sectional studies. The presence of publication bias, for example, could in theory explain the fact that heterogeneity was limited to the smallest studies, which happened to be case-control studies with physician-diagnosed asthma.

In conclusion, the evidence reviewed in this article does not support a substantial role of GST genes alone in the development of asthma. However, given the potential for interactions between GST genes and environmental toxins known to cause oxidative damage to the lungs, future research should be planned to explore gene-environment interactions. Large studies with accurate measurement of the environmental exposure are needed in order to reach adequate power to detect such interactions. Similarly, further research should evaluate possible interactions between GST genes and other genes involved in the antioxidant pathway. Future studies will also need to improve their methodological quality and the reporting of their findings, in order to increase the credibility of the evidence accumulating over time.

**Supplementary data**

Supplementary data are available at IJE online.

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**Conflict of interest:** None declared.

**KEY MESSAGES**

- Oxidative stress is involved in the pathogenesis of asthma, and glutathione-S-transferase (GST) enzymes may therefore influence asthma risk.
- Common polymorphisms of the GSTM1, GSTTI and GSTP1 genes have been associated with asthma in children and adults, but published results are inconsistent across studies.
- Our meta-analyses do not support a substantial role of the GST genes alone in the development of asthma, and they suggest presence of publication bias.
- Future studies should focus on possible interactions of the GST genes with environmental oxidative exposures and with other genes involved in the antioxidant pathway.
References


GLUTATHIONE-S-TRANSFERASE GENES AND ASTHMA PHENOTYPES


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### Appendix 1

WinBUGS code for the genetic model-free approach

**Model**

```plaintext
for(i in 1:17) {
  case[i,1:3] ~ dmulti(p.case[i,], N.case[i])
  p.case[i,1] <- 1/(1+theta[i,1]+theta[i,2])
  p.case[i,2] <- theta[i,1]/(1+theta[i,1]+theta[i,2])
  p.case[i,3] <- theta[i,2]/(1+theta[i,1]+theta[i,2])

  theta[i,1] <- beta[i,1]/C3 * exp(lambda/C3 * delta[i])
  theta[i,2] <- beta[i,2]/C3 * exp(delta[i])
  delta[i] ~ dnorm(d, prec)
}
```

```plaintext
d ~ dnorm(0.0,1.0E-6)
lambda ~ dbeta(0.5,0.5)
prec <- 1/var
var <- pow(sd,2)
sd ~ dnorm(0.1,1.0)
OR_Gg <- exp(lambda*d)
OR_GG <- exp(d)
```