

## GENETIC EPIDEMIOLOGY

# 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genes and asthma phenotypes: a Human Genome Epidemiology (HuGE) systematic review and meta-analysis including unpublished data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of the effects of *GST* genes on asthma, wheezing and bronchial hyper-responsiveness (BHR), with inclusion of unpublished data from three studies, including the large Avon Longitudinal Study of Parents and Children (ALSPAC).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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and *GSTP1* Val allele with wheezing and a negative association of *GSTP1* Val allele with BHR.

**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.<sup>1</sup> 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.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4-7</sup> The Val allele of the *GSTP1* Ile105Val polymorphism, although associated with reduced glutathione activity,<sup>8</sup> has surprisingly been reported to be highly protective for asthma and airway responsiveness in adults.<sup>5,9,10</sup> However, the Val105 allele has also been reported to increase the risk of asthma<sup>11</sup> and to increase susceptibility to the effects of ozone on breathing difficulties in children with asthma.<sup>12</sup> Others have reported no associations between this *GSTP1* polymorphism and asthma in children and adults.<sup>4,8,13</sup> 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.<sup>14,15</sup> 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 report of doctor diagnosis of asthma ever together with reported asthma, wheeze or asthma treatment in the previous 12 months. Wheezing was defined 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?'. 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 Expiratory Volume in 1 s (FEV<sub>1</sub>) at a cumulative dose of methacholine ≤1.2 mg. In the mothers' population, asthma was defined as a positive response to the question 'Have you ever had asthma?',

from a self-completion questionnaire administered during pregnancy.

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.<sup>16</sup> *GSTP1* polymorphism Ile105Val was determined using competitive allele specific polymerase chain reaction (PCR) system (KASPar). Copy number variation (CNV) of *GSTT1* and *GSTM1* deletion polymorphisms was analysed using a real-time PCR method.<sup>17</sup> 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). 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.<sup>18,19</sup> 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. *GSTM1* and *GSTT1* gene deletions were detected by multiplex PCR, with a method modified from Arand and coll<sup>20</sup> (Supplementary Appendix 1 available as supplementary data at *IJE* online). *GSTP1* polymorphism Ile105Val was determined using pyrosequencing (Biotage, Uppsala, Sweden). All assays were performed blind to the outcome status of the children.

*GSTM1* and *GSTT1* genetic effects were evaluated assuming a recessive model (OR for the null

genotype), whereas the genetic effect of *GSTP1* 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.<sup>21</sup> Inclusion criteria required two siblings (5–21 years) diagnosed with asthma and currently using asthma medication. Reported here are the associations of *GSTM1* and *GSTT1* 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<sub>1</sub> ≥70% predicted.

DNA from blood samples was analysed for CNV of *GSTM1* and *GSTT1* 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.<sup>22</sup>

#### **Systematic review**

This systematic review was conducted following a protocol in accordance with the Human Genome Epidemiology Network (HuGENet) guidelines.<sup>23</sup> 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<sup>23</sup> and STrengthening the REporting of Genetic Association studies (STREGA) recommendations for the reporting

of genetic association studies.<sup>24</sup> We also assessed the epidemiological credibility of the results of our meta-analyses by use of an 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.<sup>25</sup>

### 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.<sup>26</sup> 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.<sup>27</sup> 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.<sup>28</sup> 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 outcomes 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.<sup>29</sup> 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.<sup>30</sup> 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<sup>31,32</sup> and in a White, non-Hispanic US population.<sup>33</sup> 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**

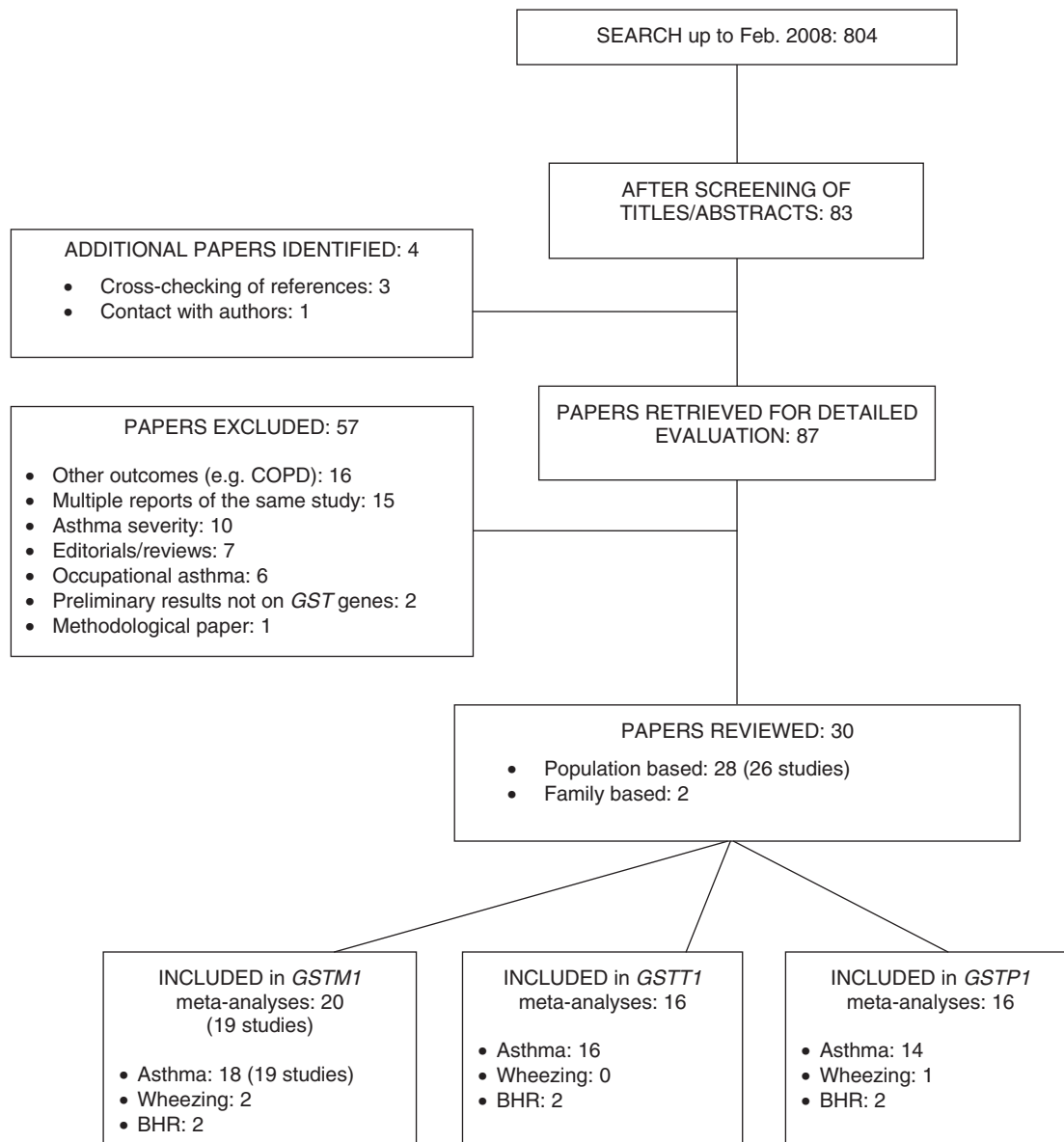
Among the 341 families, the analyses on asthma included 199 and 224 informative families for *GSTM1* and *GSTT1*, respectively; these figures were 206 and 227 for bronchial responsiveness. No association was found for asthma or bronchial responsiveness with either *GSTM1* or *GSTT1*, although the

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 FEV<sub>1</sub> reduction of  $\geq 20\%$  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

Study, year	Study population	Location, ethnicity	Adults/children	Sample size <sup>a</sup>	Definition of disease outcome
<b>Case-control studies (n = 21)</b>					
Fryer, 2000 <sup>9</sup>	Cases: from patient database Controls: healthy volunteers	UK, European	Adults	171	<b>Atopic asthma</b> 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 (>15% change in FEV <sub>1</sub> ); and positive atopic status) <b>BHR</b> FEV <sub>1</sub> reduction >20% after methacholine]
Chung, 2002 <sup>59</sup>	Cases and controls: from cohort of civil servants and their families	Korea, Asian	N/A	99	<b>Asthma</b> diagnosed by a physician (Criteria: symptoms of wheezing/ chest tightness and FEV <sub>1</sub> reduction >20% after methacholine challenge)
Freidin, 2002 <sup>60</sup>	Cases: hospitalized patients Controls: healthy volunteers	Russia, Asian	N/A	126	<b>Atopic asthma</b> diagnosed by a physician (Criteria: N/A)
Sideleva, 2002 <sup>61</sup>	Cases: hospitalized patients Controls: healthy volunteers	Russia, European	Both	199	<b>Atopic asthma</b> diagnosed by a physician (Criteria: the commonly accepted clinical laboratory examination and analysis of the external respiration function <sup>62</sup> )
Vavilin, 2002; <sup>39</sup> Safronova, 2003 <sup>38</sup>	Cases: hospitalized patients Controls: healthy volunteers	Russia, European	Children	237	<b>Asthma</b> diagnosed by a physician (Criteria: N/A)
Aynacioglu, 2004 <sup>41</sup>	Cases: consecutive asthma outpatients Controls: healthy volunteers	Turkey, Asian	Adults	475	<b>Asthma</b> diagnosed by a physician (Criteria: ECRHS protocol; medical history, physical examination, lung function tests, chest X-rays, SPT, TOT IgE)
Saadat, 2004 <sup>6</sup>	Cases: N/A Controls: healthy volunteers	Iran, Asian	Both	170	<b>Asthma</b> diagnosed by a physician [Yes to two or more criteria: (i) history of wheezing/ breathlessness; (ii) airflow obstruction of >15% FEV <sub>1</sub> ; and (iii) PEF >20%]
Tamer, 2004 <sup>11</sup>	Cases: hospitalized patients Controls: healthy volunteers	Turkey, Asian	Adults	204	<b>Asthma</b> diagnosed by a physician (Criteria: American Thoracic Society statement <sup>63</sup> )
Zhang, 2004 <sup>36</sup>	Cases: hospitalized patients Controls: N/A	China, Asian	Adults	120	<b>Asthma</b> diagnosed by a physician (Criteria: N/A)

(Continued)

Table 1 Continued

Study, year	Study population	Location, ethnicity	Adults/children	Sample size <sup>a</sup>	Definition of disease outcome
Lee, 2005, 2008 <sup>10,64</sup>	Cases: school children from three communities Controls: healthy random sample from same three communities	Taiwan, Asian	Children	397	<b>Asthma</b> parent-reported (positive response to the question, 'Has a physician ever diagnosed your child as having asthma?') <b>Wheezing</b> parent-reported (lifetime history)
Nickel, 2005 <sup>13</sup>	Cases: from Multicenter Allergy Study Controls: healthy volunteers	Germany, European	Children	205	<b>Asthma</b> diagnosed by a physician (Criteria: one or more episodes of wheezing during previous 12 months)
Oh, 2005 <sup>37</sup>	Cases: recruited at an allergy clinic Controls: healthy subjects	Korea, Asian	Both	273	<b>Asthma</b> diagnosed by a physician (Criteria: N/A)
Arbag, 2006 <sup>65</sup>	Cases: cases of nasal polyposis Controls: healthy individuals from same geographic location and same ethnicity as cases	Turkey, Asian	Both	133	<b>Asthma</b> diagnosed by a physician (Criteria: N/A; cases were receiving regular inhaled corticosteroids and beta2-adrenoreceptor stimulants for use as required)
Ercan, 2006 <sup>1</sup>	Cases: random sample from cohort of asthmatic children Controls: healthy school children	Turkey, Asian	Children	563	<b>Asthma</b> 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 FEV <sub>1</sub> following bronchodilator administration, therapeutic response to anti-asthma treatment, or an abnormal result in methacholine bronchoprovocation test (PC <sub>20</sub> <8 mg/dl)]
Holla, 2006 <sup>66</sup>	Cases and controls are unrelated subjects selected from questionnaires	Czech Republic, European	Both	637	<b>Asthma</b> diagnosed by a physician (Criteria: asthma symptoms and use of antiasthma medication according to the GINA guidelines <sup>67</sup> )
Plutecka, 2006 <sup>35</sup>	Cases: N/A Controls: healthy volunteers	Poland, European	Adults	496	<b>Asthma</b> not defined
Abdel-Alim, 2007 <sup>40</sup>	Cases: children attending outpatient clinic of allergy and immunology Controls: matched healthy children enrolled in this work	Egypt, North African	Children	90	<b>Asthma</b> diagnosed by a physician (Criteria: GINA guidelines <sup>68</sup> )
Hanene, 2007 <sup>5</sup>	Cases and controls from a representative region	Tunisia, North African	Children	347	<b>Asthma</b> not defined
Kamada, 2007a <sup>69</sup>	Cases: recruited from a medical centre Controls: healthy volunteers	Japan, Asian	Both	980	<b>Asthma</b> diagnosed by a physician (Criteria: American Thoracic Society <sup>70</sup> )
Kamada, 2007b <sup>69</sup>	Cases: recruited from a medical centre Controls: healthy volunteers	Japan, Asian	Children	289	<b>Asthma</b> diagnosed by a physician (Criteria: American Thoracic Society <sup>70</sup> )
Mak, 2007 <sup>8</sup>	Cases: recruited from asthma clinic Controls: random healthy subjects from Hong-Kong population	China, Asian	Adults	626	<b>Asthma</b> diagnosed by a physician (Criteria: cough/wheeze/chest tightness and airflow obstruction of ≥15% FEV <sub>1</sub> )
<b>Cohort studies (n = 5)</b>					
Imboden, 2007 <sup>71</sup>	SAPALDIA—a prospective multicentre study on adult Swiss general population, investigating environmental and genetic effects on lung	Switzerland, European	Adults	4422	<b>Asthma</b> self-reported (positive response to 'Have you ever had asthma?' and 'Was this confirmed by a doctor?') <b>BHR</b> FEV <sub>1</sub> reduction >20% after methacholine

(Continued)

Table 1 Continued

Study, year	Study population	Location, ethnicity	Adults/children	Sample size <sup>a</sup>	Definition of disease outcome
Schroer 2009 <sup>42</sup>	CCAAPS study on allergy and air pollution in infants with at least one atopic parent	US, mixed ethnicity	Children	498	<b>Wheezing</b> parent-reported (parental report of the child wheezing at least once at 2 years in last 12 months)
ALSPAC mothers	ALSPAC—a UK population-based birth cohort, with pregnant women recruited in 1990	UK, European	Adults	7262	<b>Asthma</b> self-reported (history of asthma reported in a questionnaire administered during pregnancy)
ALSPAC children	ALSPAC—children recruited at birth and followed up to investigate their health, behaviour and development	UK, European	Children	5330	<b>Asthma</b> parent-reported (positive response to 'Has a doctor ever said that your child has asthma?' at 7 years) <b>Wheezing</b> parent-reported (current history) <b>BHR</b> FEV <sub>1</sub> reduction >20% after methacholine
AMICS-INMA	AMICS—a population-based birth cohort included in the Spanish environment and childhood research network (INMA study)	Spain, European	Children	428	<b>Asthma</b> 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) <b>Wheezing</b> parent-reported (current wheeze at 6 years)
<b>Cross-sectional studies (n = 2)</b>					
Salam 2007; <sup>7</sup> Gilliland, 2002 <sup>33</sup>	Cross-sectional study as part of the Children Health Study, a Californian ongoing cohort study of school children in 12 communities	US, Mixed ethnicity	Children	3081	<b>Asthma</b> parent-reported DDA (Criteria: N/A) <b>Wheezing</b> parent-reported (lifetime history)
Kabesch 2004 <sup>53</sup>	Cross-sectional study part of ISAAC project, assessing prevalence of asthma and allergy in school children	Germany, European	Children	3005	<b>Asthma</b> parent-reported DDA (Criteria: N/A)
<b>Family studies (n = 3)</b>					
David 2003 <sup>34</sup>	Family study based on case-parent triad design and focused on gene-environment interactions	Mexico, Latin American	Children	218 families	<b>Asthma</b> diagnosed by a physician (Criteria: included skin-prick and pulmonary function testing)
Brasch-Andersen 2004 <sup>4</sup>	Family study that recruited atopic families with asthmatic children in two separate samples. Sample A: asthma; Sample B: atopic asthma	Denmark, European	Children	246 families	(Sample A) <b>Asthma</b> diagnosed by a physician (Criteria: clinical symptoms plus positive methacholine challenge) (Sample B) <b>Atopic asthma</b> diagnosed by a physician (Criteria: clinical symptoms plus positive specific IgE measurement)
Southampton	UK multicentre family study that recruited families with two siblings diagnosed with asthma	UK, European	Children and young adults	341 families	<b>Asthma</b> 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') <b>Bronchial responsiveness</b> methacholine dose response measure

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

<sup>a</sup>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 2** Summary of genotype frequencies by disease status in the population-based studies

Study, year	GSTMI (null/present) [CNV: 0/1/2 copies]		GSTT1 (null/present) [CNV: 0/1/2 copies]		GSTP1 (Ile105Ile/Ile105Val/Val105Val)		Disequilibrium coefficient (P-value) <sup>a</sup>
	Affected	Non-affected	Affected	Non-affected	Affected	Non-affected	
<b>Asthma</b>							
Fryer, 2000 <sup>9</sup>	72/53	24/20	24/103	7/37	54/66/7	10/26/8	-0.046 (0.36)
Chung, 2002 <sup>59</sup>	16/17	31/35	21/12	34/32	25/8/0	51/15/0	-0.013 (0.59)
Freidin, 2002 <sup>60</sup>	52/17	42/15	24/45	15/42			
Sidleva, 2002 <sup>61</sup>	83/26	43/47	73/36	21/69			
Vavilin, 2002 <sup>39</sup>	52/48	44/60	26/74	12/92	54/77/10	32/61/3	-0.091 (<0.01)
Safronova, 2003 <sup>38</sup>							
Aynacioglu, 2004 <sup>41</sup>							
Kabesch, 2004 <sup>53</sup>	148/120	1394/1343	42/226	477/2258	109/93/8	134/99/32	0.026 (0.04)
Saadat, 2004 <sup>6</sup>	42/43	20/65	55/30	35/50			
Tamer, 2004 <sup>11</sup>	64/37	42/61	27/74	25/78	33/45/23	49/46/8	-0.013 (0.64)
Zhang, 2004 <sup>36</sup>	49/11	18/42	43/17	7/53			
Lee, 2005 <sup>10</sup>	49/33	97/87			62/18/2	112/64/8	-0.004 (1.00)
Nickel, 2005 <sup>13</sup>					38/39/6	54/60/8	-0.031 (0.14)
Oh, 2005 <sup>37</sup>					102/45/7	75/39/5	-0.000 (1.00)
Arbag, 2006 <sup>65</sup>	12/19	47/55	11/20	24/78			
Ercan, 2006 <sup>1</sup>	124/179	100/151	64/246	48/202	174/116/22	128/98/25	0.013 (0.36)
Holla, 2006 <sup>66</sup>	166/140	166/165	59/247	73/258			
Plutecka, 2006 <sup>35</sup>							
Abdel-Alim, 2007 <sup>40</sup>					137/123/26	94/95/21	-0.006 (0.75)
					32/4/14	9/2/29	0.163 (<0.01)
Hanenc, 2007 <sup>5</sup>	84/37	111/115	41/80	67/159	63/45/13	78/107/41	0.007 (0.68)
Imboden, 2007 <sup>71</sup>	73/71	2249/2029	25/119	797/3481			
Kamada, 2007a <sup>69</sup>	178/189	326/287					
Kamada, 2007b <sup>69</sup>	57/57	95/80					
Mak, 2007 <sup>8</sup>	161/150	185/130	144/167	168/147	207/94/11	214/91/9	-0.001 (1.00)
Salam, 2007 <sup>7</sup>	240/215	1226/1300	91/363	537/1992	171/230/65	1043/1229/343	-0.004 (0.37)
ALSPAC children	419/333	2419/2156	124/621	786/3769	307/360/92	1985/2006/585	0.005 (0.10)
	[419/249/49]	[2419/1689/306]	[124/313/227]	[786/1917/1276]			
ALSPAC mothers	239/223	3523/3064	74/386	1069/5488	337/377/105	2684/2935/755	-0.003 (0.26)
	[239/179/28]	[3523/2388/435]	[74/164/148]	[1069/2787/1782]			

(Continued)

Table 2 Continued

Study, year	GSTM1 (null/present) [CNV: 0/1/2 copies]		GSTT1 (null/present) [CNV: 0/1/2 copies]		GSTP1 (Ile105Ile/Ile105Val/Val105Val)		Disequilibrium Coefficient (P-value) <sup>a</sup>
	Affected	Non-affected	Affected	Non-affected	Affected	Non-Affected	
AMIGS-INMA	11/7	224/169	4/14	74/319	8/8/2	177/183/33	-0.016 (0.17)
<b>Wheezing</b>							
Gilliland, 2002 <sup>33</sup>	455/529	816/1010			40/41/18	186/155/58	0.03 (0.008)
Lee, 2008 <sup>64</sup>	128/85	99/85			256/330/68	2297/2364/677	0.002 (0.41)
Schroer, 2009 <sup>42</sup>					9/20/7	174/167/27	-0.015 (0.17)
ALSPAC children	358/289	2831/2513	114/530	898/4415			
AMIGS-INMA study	19/17	211/157	7/29	71/297			
<b>BHR</b>							
Fryer 2000 <sup>9</sup>	30/22	24/20	7/46	7/37	21/29/3	10/26/8	-0.046 (0.36)
Imboden 2007 <sup>71</sup>	327/298	1995/1802	115/510	707/3090	253/266/48	1792/1650/358	-0.004 (0.17)
ALSPAC children	278/246	1515/1391	81/437	477/2407	210/251/64	1206/1321/365	-0.001 (0.85)

<sup>a</sup>HWE tested (exact test) among controls in case-control studies, and in the whole population otherwise.

results<sup>4</sup> and no meta-analysis could be performed. The study by Brasch-Andersen and colleagues,<sup>4</sup> 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,<sup>34</sup> 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 the *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 the 2 family-based articles. Three articles<sup>6,35,36</sup> did not describe the study population (selection of cases and/or controls for case-control studies), and six articles<sup>5,35-39</sup> did not provide a definition of the outcome considered (asthma in all cases). HWE was reported to be assessed in less than half of the articles (46%). 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 blinding of genotyping to the clinical status of the subjects. Among the population-based studies, 35% of the articles discussed the issue of population stratification, although only a few discussed the potential problem in some detail.

Four studies were identified as being of poor quality (no outcome definition plus incomplete description of study population/no assessment of HWE). All of them were case-control studies with total sample size less than 500, and they were published in Russian,<sup>39</sup> Chinese,<sup>36</sup> Korean<sup>37</sup> and international<sup>35</sup> journals.

Table 2 shows deviation from HWE for *GSTP1* gene in four studies of relatively small size (less than 500), including three case-control studies of rather poor quality,<sup>38,40,41</sup> and a cohort study<sup>42</sup>. Only two of them<sup>41,42</sup> performed quality control of genotyping and found no genotyping error, and none of them reported blinded genotyping. Only one of them<sup>42</sup>

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 23 902 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<sup>36,39</sup> 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 22 880 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<sup>36,39</sup> 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 14 442 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<sup>35,37</sup> 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<sup>9</sup> 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.<sup>26</sup> 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.

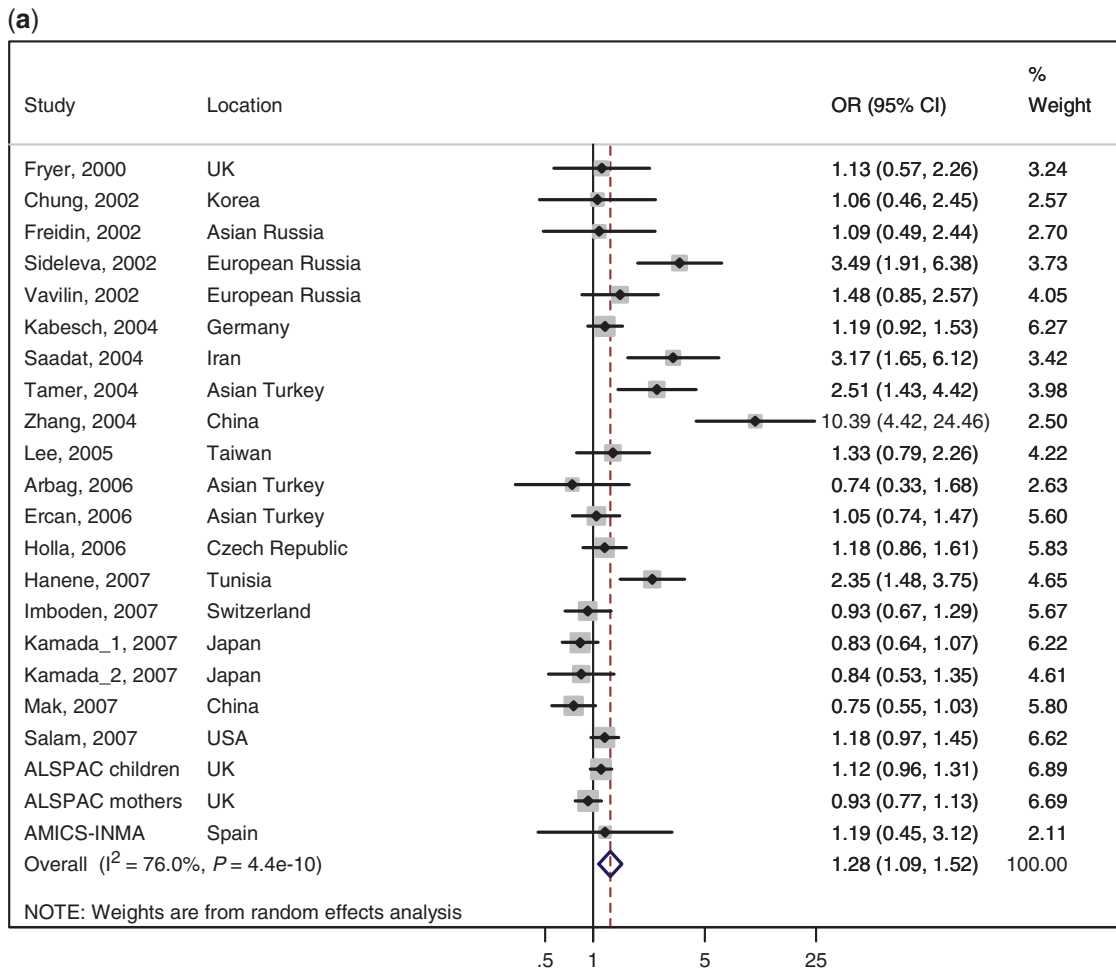
**Strength of the evidence from the meta-analyses**

When applying the Venice criteria to assess credibility,<sup>25</sup> the cumulative evidence reviewed does not clearly support a genetic effect for any of the three *GST* genes on asthma. In fact, although the amount of evidence is good for all three meta-analyses,

particularly those on *GSTM1* and *GSTT1*, 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**

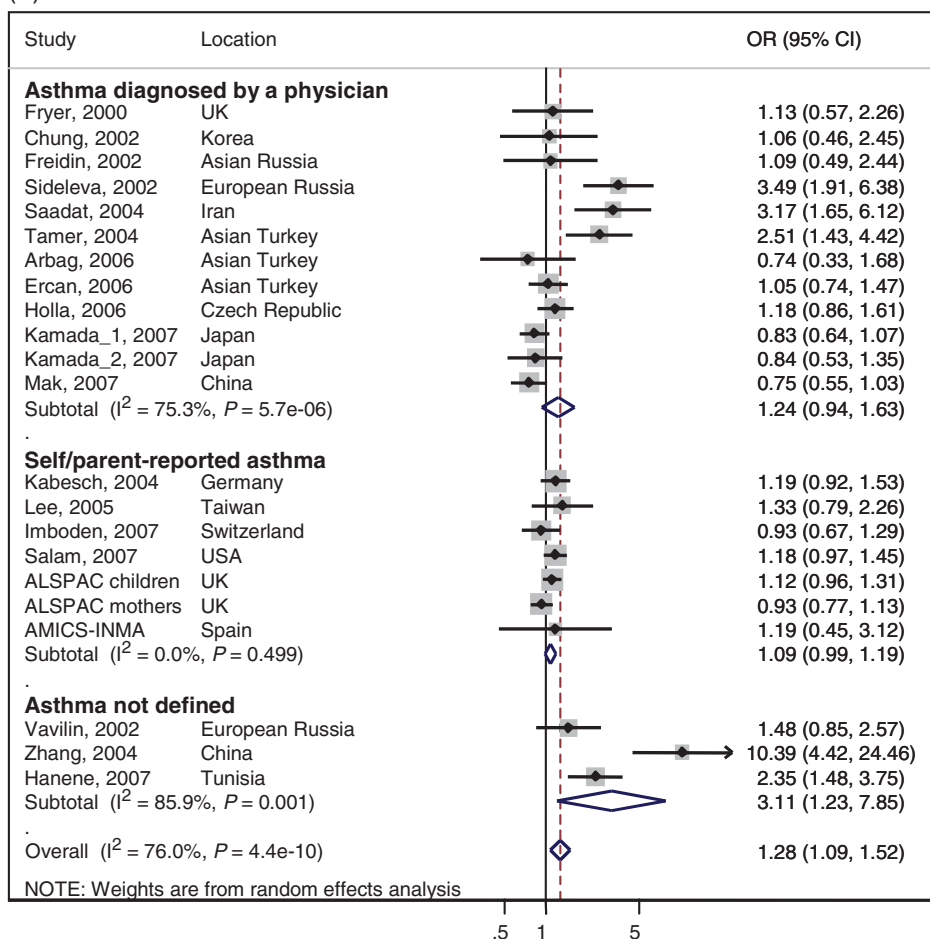
Overall, the evidence synthesized in this review does not support a substantial role of *GST* genes on asthma phenotypes in either children or adults, although small effects cannot be excluded and it is possible that these genes act on airway disease through interaction with environmental exposures or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



**Figure 2** Random effects meta-analysis of *GSTM1* (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)



(c)

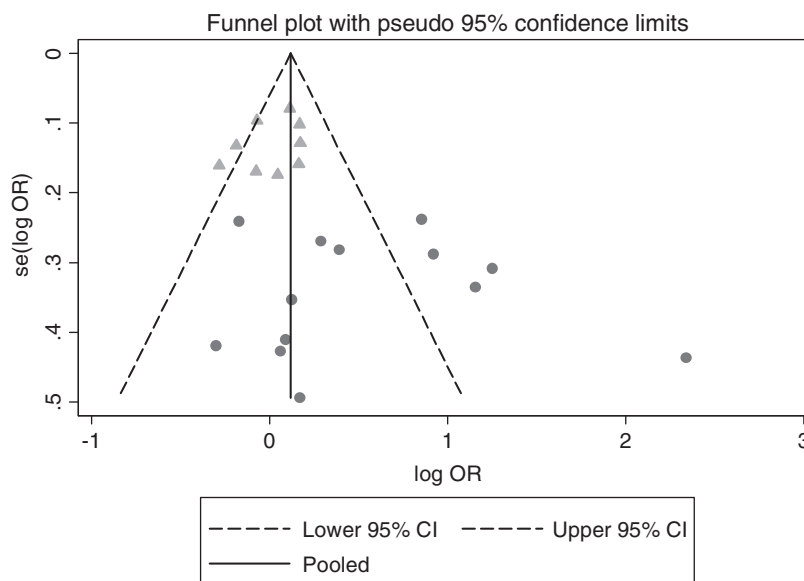
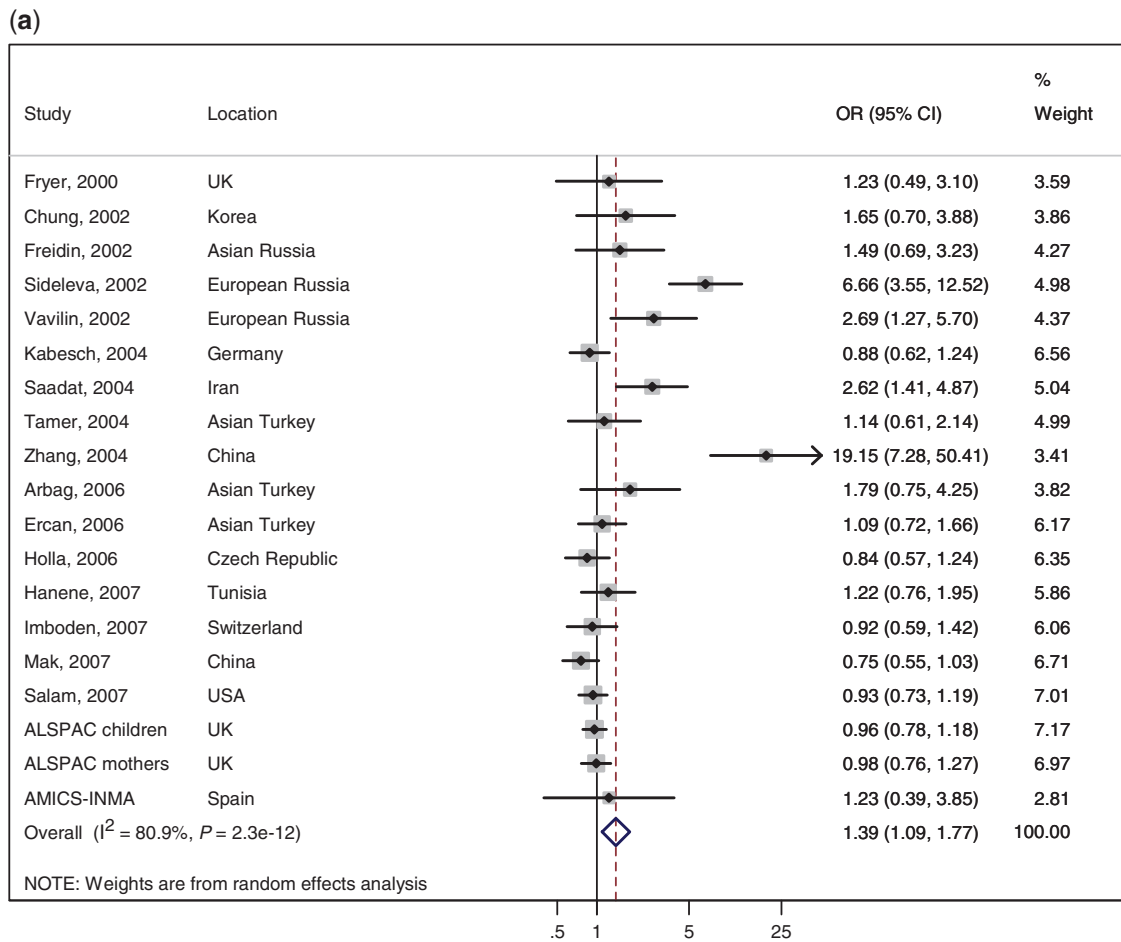


Figure 2 Continued

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.<sup>43</sup>

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** 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:  $P = 0.001$ )

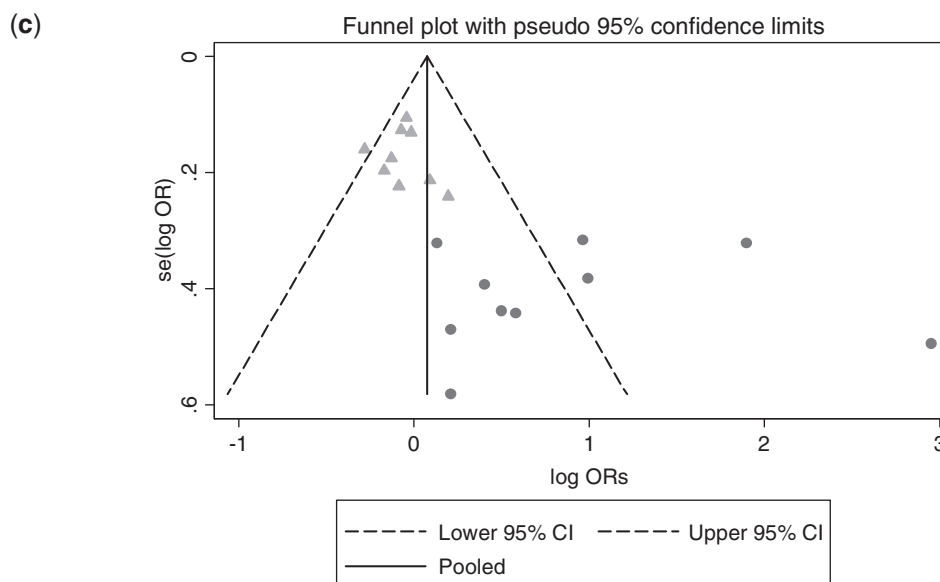
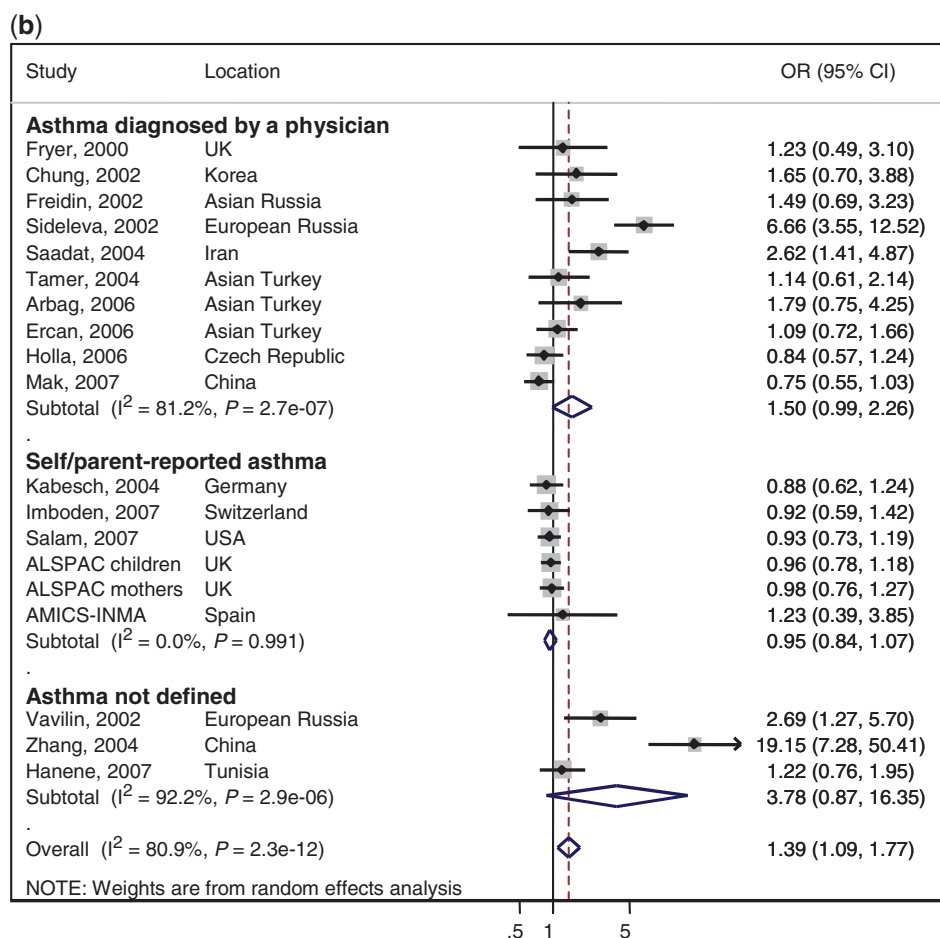
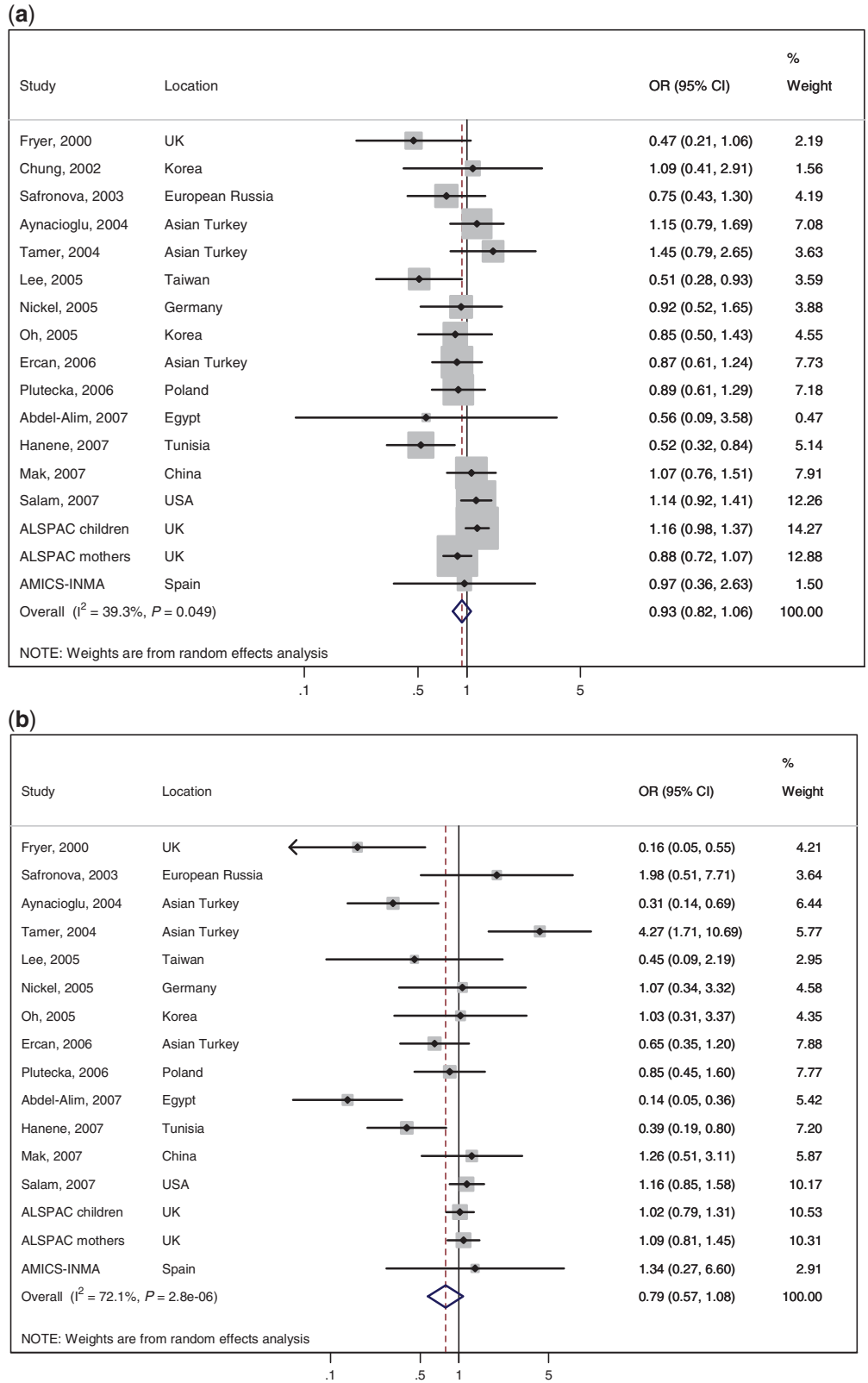


Figure 3 Continued

meta-analysis suggests a possible small effect of the null genotype in increasing the risk of ~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 *GSTT1* or *GSTM1* is



**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



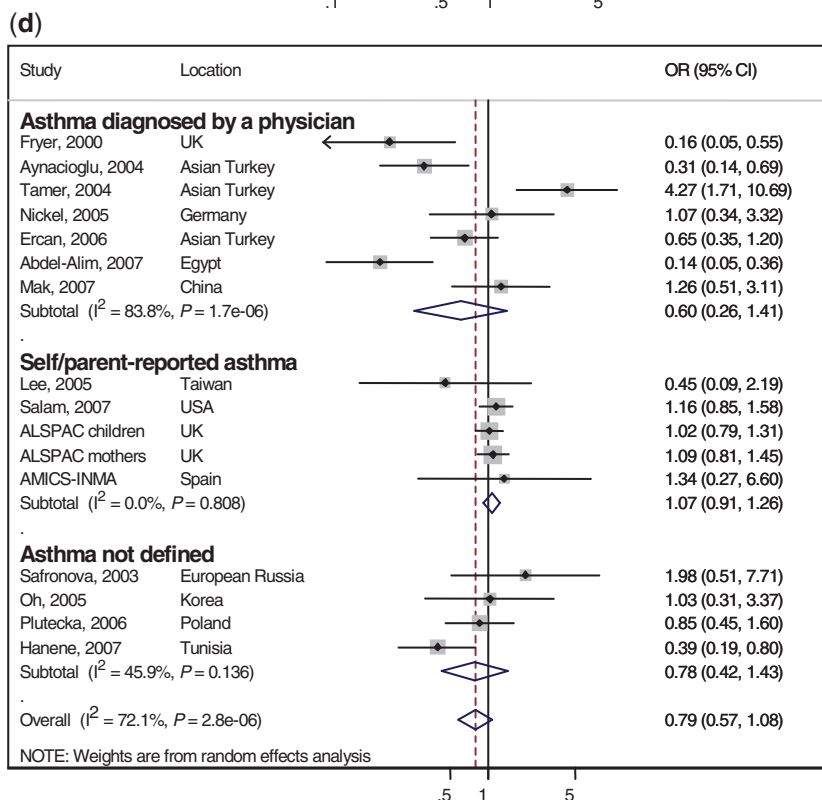
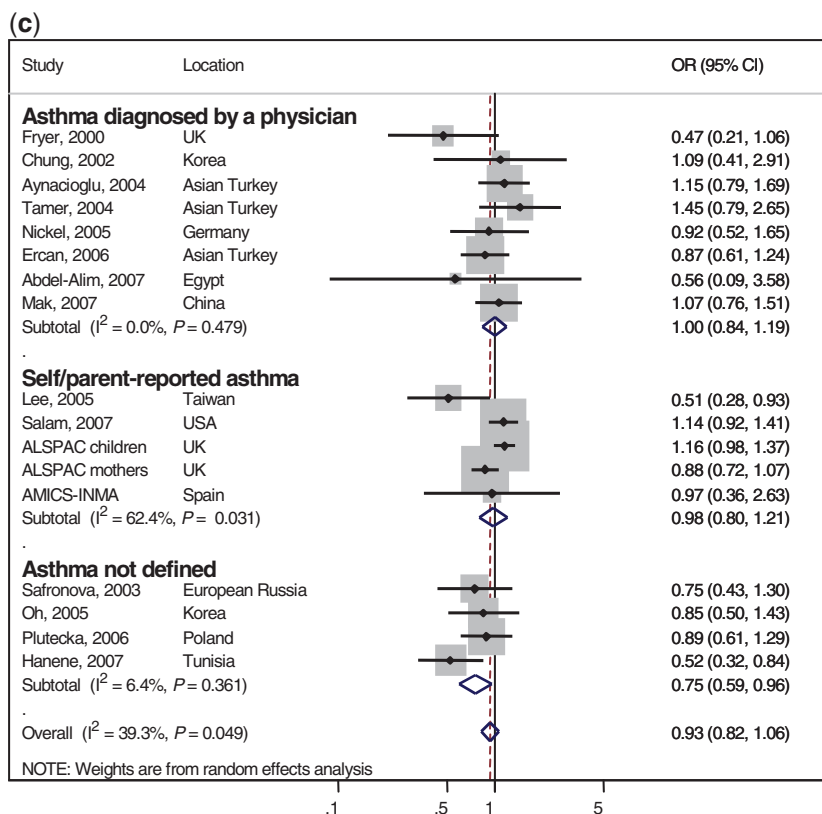
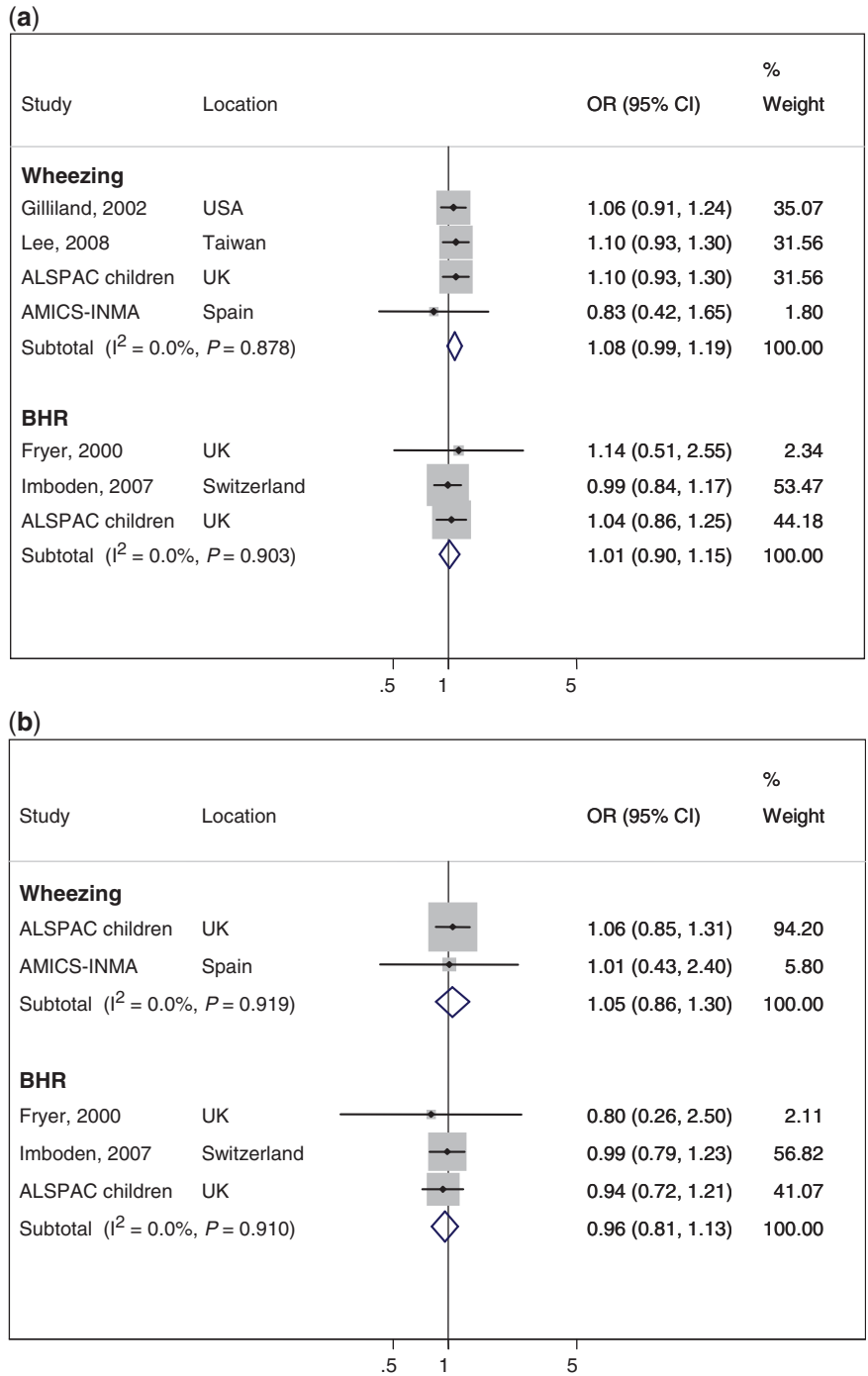


Figure 4 Continued

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.<sup>44,45</sup> 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*.<sup>4</sup> A similar approach was utilized to obtain gene-dosage information in the ALSPAC cohort.<sup>17</sup>



**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

The meta-analysis on *GSTP1* Ile105Val polymorphism and asthma suggested a possible, weak protective effect of the Val allele. The Val substitution in the *GSTP1* gene is associated with altered substrate affinities compared with the Ile105 wild-type allele<sup>46</sup> and heterozygosity has also been associated with reduced risk of chronic obstructive pulmonary disease.<sup>47</sup> Although in the meta-analysis on *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. *GST* genes effects on asthma risk may be modified by exposures that produce lung oxidative injury, such as smoking, air

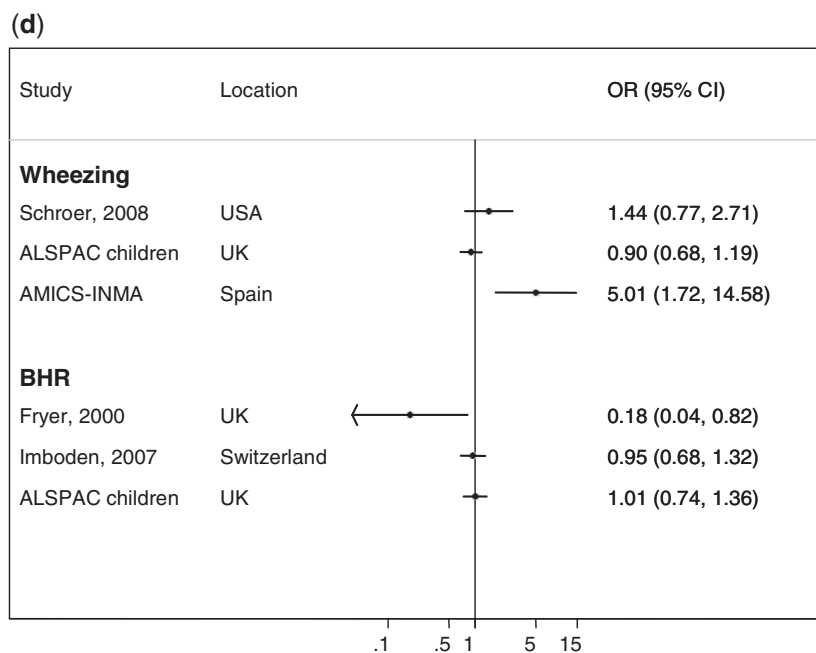
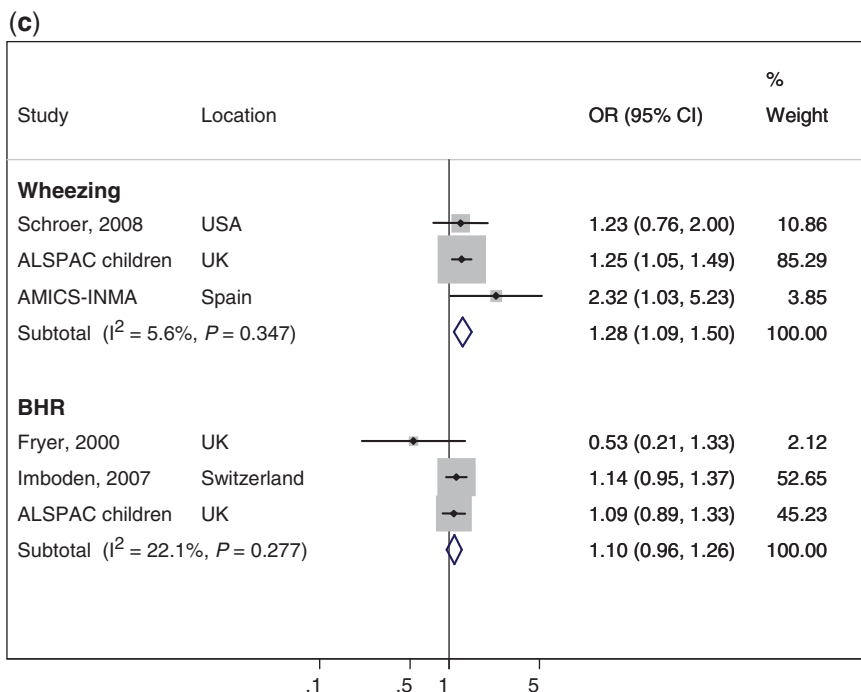


Figure 5 Continued

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.<sup>48</sup> 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.<sup>49</sup> 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.<sup>49–51</sup> 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,<sup>52</sup> 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.<sup>51</sup> Only a small proportion of the studies reviewed in this article reported on gene–environment interactions, including environmental tobacco smoke (ETS),<sup>33,39,53</sup> *in utero* ETS<sup>33,53</sup> and air pollution,<sup>7,54</sup> 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 etio-pathogenesis 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%.<sup>55</sup> 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 isoform 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.<sup>56</sup> 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.<sup>34</sup> 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.<sup>12,32,57</sup>

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.<sup>58</sup> 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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### 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*, *GSTT1* 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.

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## Appendix 1

WinBUGS code for the genetic model-free approach

### Model

```
{
  for(i in 1:17) {
    case[i,1:3] ~ dmulti(p.case[i,], N.case[i])
```

```
control[i,1:3] ~ dmulti(p.ctrl[i,], N.ctrl[i])

p.ctrl[i,1] <- 1/(1+beta[i,1]+beta[i,2])
p.ctrl[i,2] <- beta[i,1]/(1+beta[i,1]+beta[i,2])
p.ctrl[i,3] <- beta[i,2]/(1+beta[i,1]+beta[i,2])

beta[i,1] ~ dgamma(0.001,0.001)
beta[i,2] ~ dgamma(0.001,0.001)

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]*exp(lambda*delta[i])
theta[i,2] <- beta[i,2]*exp(delta[i])
delta[i] ~ dnorm(d, prec)

}

d ~ dnorm(0.0,1.0E-6)
lambda ~ dbeta(0.5,0.5)
prec <- 1/var
var <- pow(sd,2)
sd ~ dnorm(0,1)I(0,)
OR_Gg <- exp(lambda*d)
OR_GG <- exp(d)
```

```
}
```