

Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma

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Summary

Background Polymorphisms at the glutathione S-transferase (GST) P1 locus were associated with asthma-related phenotypes and bronchial hyper-responsiveness.

Objective This study investigated whether GSTP1 genotypes and outdoor air pollution were interactive risk factors on childhood asthma.

Methods Four hundred and thirty-six subjects were recruited for oral mucosa samplings from 2853 fourth- to ninth-grade schoolchildren from three districts with different air pollution levels in southern Taiwan. PCR-based assays were performed by oral mucosa DNA to determine GSTP1 genotypes. We also conducted a nested case-control study comprising 61 asthmatic children and 95 controls confirmed by International Study of Asthma and Allergies in Childhood questionnaire results and methacholine challenge test. Multiple logistic regression was used to adjust for potential confounding factors.

Results All participants were homozygous at the Ala-114 locus. Although only a marginally significant association existed between the frequency of homozygosity at the Ile-105 locus and asthma when air pollution was not considered, we found a significant gene-environmental interaction between GSTP1-105 alleles and air pollution after adjusting for confounders ($P = 0.035$). Specifically, we found that compared with participants carrying any Val-105 allele in low air pollution, those who are Ile-105 homozygotes in high air pollution district had a significantly increased risk of asthma (adjusted odds ratio (AOR) = 5.52, 95% confidence interval (CI) = 1.64–21.25). Compared with participants carrying any Val-105 allele, in high air pollution district, children with Ile-105 homozygotes had a significantly increased risk of asthma (AOR = 3.79, 95% CI = 1.01–17.08), but those who carried two Ile-105 alleles in low or moderate air pollution districts did not show similar tendencies. The risk of asthma also revealed a clear dose-response relationship with outdoor air pollution in children with Ile-105 homozygotes.

Conclusion Our result suggests a gene-environmental interaction between GSTP1-105 genotypes and outdoor air pollution on childhood asthma.

Keywords air pollution, asthma, children, gene-environmental interaction, GSTP1 polymorphism

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Introduction

Asthma is the single most common chronic childhood disease in developed nations [1]. Current studies indicate that many regions of the human genome containing susceptibility genes are associated with various asthmatic phenotypes [2, 3]. It is also believed that the inheritance of asthma does not follow a simple monogenic pattern [4, 5]. Although host genetic factors may contribute to the occurrence of childhood asthma, it is equally probable that environmental factors

have crucial interactive roles in determining whether susceptible children develop such allergic diseases.

The presence of inflammation in the airway is an important biochemical feature of asthma. Oxidative stress, with the formation of reactive oxygen species (ROS), is a key component of inflammation [6]. Studies have shown that individuals with lowered antioxidant capacity are at increased risk of asthma [7]. Inability to detoxify ROS, including lipid and DNA hydroperoxides, should perpetuate inflammatory process in the lung, activate bronchoconstrictor mechanisms, and precipitate asthmatic symptoms. Members of the glutathione S-transferase (GST) supergene family are critical for protecting cells from ROS because they can utilize as substrates a wide variety of products of oxidative stress and also influence the synthesis of eicosanoid-like mediators via modulation of ROS levels [8, 9]. In human lung epithelium,

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the GSTP1 gene contributes more than 90% of GST-derived enzyme activity [10]. This gene maps on chromosome 11q13, which was suggested as a candidate region for asthma and bronchial hyper-responsiveness (BHR) in some linkage studies [11]. Although GSTP1 in a region is associated with asthma, few data have been established for a relationship between asthma and the known sequence variants (at codons 105 and 114) in this gene [12, 13].

Recent studies have revealed that exposure to outdoor air pollutants may increase the risks of asthmatic symptoms [14–17], and that it is also associated with airway inflammation and BHR [15, 18]. Air pollution has been linked to increased hospitalizations [19], emergency room and clinic visits [20], and medication use because of exacerbation of symptoms [21] in children with asthma. Although outdoor air pollution is suggested to be a risk factor for childhood asthma, only a portion of the children who reside in areas with high air pollution have asthma. Some researchers have speculated about a genetic susceptibility to the effect of air pollution [22], but this gene–environmental interaction has not been examined between childhood asthma and outdoor air pollution.

In this report, we compared the genotype distribution of a bi-allelic polymorphism of the GSTP1 gene in a case–control study nested within our previous investigation of asthma in Taiwanese schoolchildren [23]. We also examined the association of childhood asthma with GSTP1 gene polymorphisms and outdoor air pollution, and evaluated their interactions.

Materials and methods

Study design

In 2001, we conducted a national, cross-sectional, school-based survey for respiratory diseases and symptoms in middle and elementary schoolchildren. The study protocol has been described previously [23]. Briefly, the standard 'International Study of Asthma and Allergies in Childhood'-Chinese version (ISAAC-C) questionnaire was taken home by students and answered by parents. Some additional information concerning basic demography and residential factors was also collected in the questionnaire survey. The schools chosen were within 1 km catchment areas of monitoring stations of the Taiwan Environmental Protection Agency (EPA). Stratified sampling by grade was applied in each school.

Complete monitoring data for the air pollutant criteria were available from EPA monitoring stations beginning in 1994. Six schools from three districts with monitoring stations in southern Taiwan, Sanmin (high air pollution level), Annan (moderate air pollution level), and Singang (low air pollution level), were included in this study because their air-monitoring data fit the tertile levels of air pollutant criteria, nitrogen oxides (NO_x) and sulphur dioxides (SO_2), in recent years. From 1994 to 2001, mean NO_x and SO_2 concentrations were 41.2 and 8.6 parts per billion (ppb) for Sanmin, 26.2 and 6.2 ppb for Annan, and 22.6 and 3.6 ppb for Singang, respectively. Trends of annual mean concentration of NO_x and SO_2 were shown between 1994 and 2001 (Fig. 1).

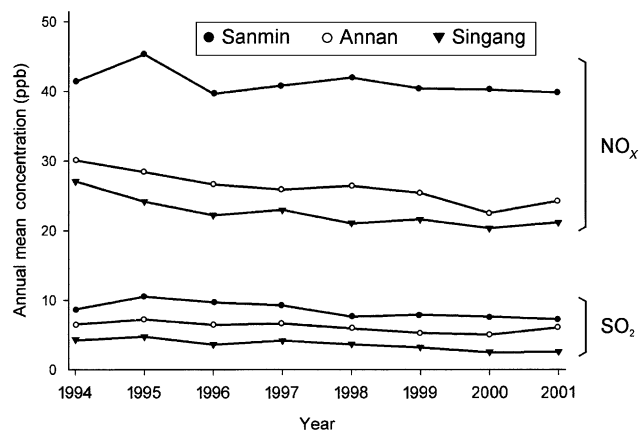


Fig. 1. Trends of nitrogen oxides (NO_x) and sulphur dioxides (SO_2) in Sanmin, Annan, and Singang from 1994 to 2001.

Subject selection

The definition of asthma cases in this study was determined by a positive response to the question, 'Has a physician ever diagnosed your child as having asthma?' or 'Has your child ever had dyspnea with wheezing in the chest at any time in the past?' Five affiliated questions concerning current asthmatic symptoms were also asked:

1. In the past 12 months, has your child had dyspnea with wheezing in the chest? (Wheeze)
2. In the past 12 months, has your child's sleep been disturbed because of wheezing? (Night wheeze)
3. In the past 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths? (Dyspnea at rest)
4. In the past 12 months, has your child's chest sounded wheezy during or after exercise? (Exercise wheeze)
5. In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection? (Night cough)

There were totally 2853 fourth- to ninth-grade schoolchildren from the three districts who completed the questionnaire survey. In June 2001, we chose 30% asthmatic children and 8% controls for the present study based on criteria established from questionnaire information. Four hundred and thirty-six subjects were recruited for oral mucosa samplings and pulmonary function tests. All of the selected children were lifelong non-smokers and were of the same ethnic origin. Standardized pulmonary function tests were conducted with equipment that met American Thoracic Society criteria (Model 2130; SensorMedics, Yorba Linda, CA, USA) [24], and the manoeuvres were performed in a standardized manner [25]. Quality control consisted of a 3 L syringe calibration and a leak test and ambient temperature and atmospheric pressure were measured before the test. Every procedure was executed by the same group of fieldworkers in a double-blinded manner. Methacholine challenge tests were performed on subjects who fitted the following criteria [26]: (1) baseline forced expiratory volume in 1 s (FEV_1) $\geq 70\%$ of predicted value; (2) not suffered a viral infection or common flu within at least 2 weeks; (3) no medication or herb drugs use within at least 1 week preceding

the study; and (4) informed consent was obtained from the parent. Of 436 subjects, 247 completed the methacholine challenge test and nine individuals with asthmatic history did not complete tests because of obvious wheezing and dyspnea, although 20% decline in FEV₁ was not reached. The study protocol was approved by the Institutional Review Board at our university hospital and it complied with the principles outlined in the Helsinki Declaration [27].

We selected participants with asthma using the following criteria: (1) physician-diagnosed asthma or dyspnea with wheezing and (2) PD₂₀ < 2 mg. Only 55 children met these criteria and were included in this report. Six asthmatic children who responded with obvious wheezing and dyspnea to the final methacholine challenge dose of < 2 mg were added despite their not having completed the tests. Ninety-five children met the criteria for controls: (1) no physician-diagnosed asthma or dyspnea with wheezing in the past; (2) no positive response to any of the five questions concerning current asthmatic symptoms; (3) negative methacholine challenge test; (4) baseline FEV₁ > 90% of predicted value; and (5) FEV₁/forced vital capacity (FVC) ≥ 80%. Table 1 provides a summary of criteria for selection of participants with asthma and controls.

Glutathione S-transferase P1 gene polymorphism genotyping

Cotton swabs containing oral mucosa were collected and were immediately maintained at -80 °C throughout the transfer and storage. Genomic DNA was isolated using phenol/chloroform extraction method previously described [28] with some modification. In brief, the cotton swabs were directly immersed in 300 µL cell lysis buffer (50 mM Tris-HCl, 1 mM EDTA, 0.1 M NaCl, pH 8.0) containing 2% SDS and 20 µg/mL proteinase K in a 1.5 mL microcentrifuge tube. After incubation overnight at 55 °C, the swabs were discarded and the DNA in supernatants was purified by phenol/chloroform extraction and then precipitated with ethanol.

GSTP1 gene variants are caused by base-pair transitions at nucleotides +313 and +341 [12, 13]. We detected the polymorphism at nucleotide +313 by PCR with the primers 5'-CTCTATGGGAAGGACCAGCAGGAG-3' and 5'-CA-

AGCCACCTGAGGGGTAAGG-3', and digestion of the product with *Alw26I*. The polymorphism at nucleotide +341 was detected by PCR with the primers 5'-GTTGTG-GGGAGCAAGCAGAGG-3' and 5'-CACAAATGAAGG-TCTTGCCTCCC-3', and digestion of the product with *AclI*. The digested PCR products were analysed by 2.5% agarose gel electrophoresis. PCR condition: the 50 µL PCR mixture containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 µM dNTP, 0.2 µM each primer, 2 U Taq DNA polymerase, and 20 ng genomic DNA was amplified by 40 cycling reactions composed of 95 °C denaturation for 30 s, 60 °C annealing for 1 min, and 72 °C elongation for 1 min. All assays were performed by workers unaware of the clinical status of individual subjects, and genotype assignments were based on two consistent experimental results. About 15% of randomly selected samples were directly sequenced and all of them were concordant with the initial genotyping results.

Statistical analysis

We first examined the frequency distribution of GSTP1 genotypes by case-control status by χ^2 tests. To assess the presence of gene-environmental interactions between the GSTP1-105 polymorphism and air pollution, we compared the risk of asthma for subjects in each category of joint exposure with that of subjects who were Ile-Val or Val-Val for the GST-105 polymorphism and who had the lowest air pollution level. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated from unconditional logistic regression models with adjustment for potential confounders. Individual and joint associations were estimated using indicator variables created for each category, omitting the hypothesized low-low risk category or reference group. For categorical variables with more than two categories, the interaction was evaluated using the likelihood ratio test (LRT), comparing the model with indicator variables for the cross-classified variables with a reduced model containing indicator variables for the main effects only [29]. In addition, separate analyses were conducted for relationship between questionnaire-defined asthma and air pollution by GSTP1-105 genotype. Statistical significance was set at $P < 0.05$ based on a two-sided calculation.

Results

Our study finally comprised 61 participants with asthma and 95 controls from three districts in southern Taiwan. The overall allele frequencies of the two polymorphisms were as follows: (a) Ile-105 = 78.2%/Val-105 = 21.8% and (b) Ala-114 = 100%/Val-114 = 0%. Table 2 presents the demographic, lung function, air pollution of the district, and genotype data by children with asthma and controls. Children with asthma were slightly younger, included a higher proportion of girls than did the controls, tended to have more visible cockroaches or incense exposure at home, and were more likely to live in areas with higher levels of air pollution. As expected, children with asthma had reduced baseline FEV₁/FVC and a lower percentage of predicted FEV₁.

Table 1. Criteria for selection of asthmatic children and controls

Criteria	Asthmatic children	Controls
Physician-diagnosed asthma or dyspnea with wheezing in the past	Yes	No
Symptoms in the past 12 months		
Wheeze	Yes/no	No
Night wheeze	Yes/no	No
Dyspnea at rest	Yes/no	No
Exercise wheeze	Yes/no	No
Night cough	Yes/no	No
Percent of predicted FEV ₁	≥ 70%	> 90%
FEV ₁ /FVC	Not limited	≥ 80%
PD ₂₀ in methacholine challenge test	< 2 mg	No response*

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

*Did not respond to methacholine challenge tests at the highest dose of 4.7 mg.

All participants in this study were homozygous at the Ala-114 locus. Children with asthma were prone to have a higher percentage of the Ile-105/Ile-105 genotype and a lower

Table 2. Demographic, phenotypic, and genotypic characteristics in asthmatic children and controls

Categories	Asthmatic children (<i>n</i> = 61)	Controls (<i>n</i> = 95)
Age (year)	11.8 ± 1.5	12.1 ± 1.8
Sex		
Boys	33 (54.1)	54 (56.8)
Girls	28 (45.9)	41 (43.2)
Percent of predicted FEV ₁ (%)*	90.3 ± 10.2	102.2 ± 10.4
FEV ₁ /FVC (%)*	87.0 ± 8.9	92.1 ± 5.7
PD ₂₀ in methacholine challenge test (mg)	1.06 ± 0.58†	NA
Air pollution of the district		
High	27 (44.3)	32 (33.7)
Median	23 (37.7)	30 (32.6)
Low	11 (18.0)	33 (34.7)
Incense burning at home	30 (49.2)	41 (43.2)
Cockroaches seen at home	48 (78.7)	72 (75.8)
GSTP1-105‡		
Ile-Ile	43 (70.5)	52 (54.7)
Ile-Val	16 (26.2)	38 (40.0)
Val-Val	2 (3.3)	5 (5.3)
GSTP1-114		
Ala-Ala	61 (100)	95 (100)

Results shown as mean ± SD or *n* (%).

NA, not available because some participants did not respond at the highest dose; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GST, glutathione S-transferase.

**P* < 0.01. All others showed no significant difference between two groups.

†PD₂₀ did not include those who did not complete methacholine test because of obvious wheezing and dyspnea.

‡Hardy-Weinberg equilibrium tests showed insignificance (*P* > 0.05) in both asthma and control groups.

percentage of the Ile-105/Val-105 or Val-105/Val-105 genotypes than controls. Because distributions of the Ile-105/Val-105 and Val-105/Val-105 genotypes between children with asthma and controls were similar, and the frequency of homozygosity at the Val-105 locus was relatively low, we combined the Ile-105/Val-105 and Val-105/Val-105 genotypes in the subsequent analyses. We performed a χ^2 test to compare the GSTP1-105 genotype distribution of asthma cases vs. the control subjects. The result was not statistically significant (χ^2 (d.f., 2) = 3.941, *P* = 0.139). After we combined the Ile-105/Val-105 and Val-105/Val-105 genotypes, the χ^2 result appeared marginally significant (χ^2 (d.f., 1) = 3.938, *P* = 0.047).

We also examined the distribution of GSTP1 gene polymorphisms at codon 105 in asthmatic children and controls by air pollution level (high, moderate, and low) in districts (Table 3). In the low air pollution district, the frequency of Ile-105 homozygosity was not significantly related to an increased risk of childhood asthma (adjusted OR (AOR) = 1.43, 95% CI: 0.34–6.25). In the moderate and high air pollution districts, the risk of asthma significantly increased for individuals carrying two Ile-105 alleles (AOR = 4.14 in moderate air pollution, and AOR = 5.52 in high air pollution). In order to evaluate genetic effects in different outdoor air pollution levels, we restricted our logistic regression analyses to moderate and high air pollution districts. Using Ile/Val or Val/Val of the GSTP1-105 polymorphism in certain air pollution levels as a reference category, the AOR for asthma was 1.54 (95% CI: 0.41–5.97) in the moderate air pollution district and was 3.79 (95% CI: 1.01–17.08) in the high air pollution district (Table 4). After adjustment for potential confounders, the test for interaction between the GSTP1-105 genotype and air pollution by LRT gave us a χ^2 (d.f., 2) of 6.729, which corresponded to a statistical significance (*P* = 0.035).

Table 3. ORs for asthma with GSTP1-105 genetic polymorphism by different air pollution levels

GSTP1-105 polymorphism	District air pollution level	Asthmatic children (<i>n</i>)	Controls (<i>n</i>)	Crude OR	95% CI	AOR*	95% CI
Ile-Val or Val-Val	Low	5	16	1.00		1.00	
Ile-Ile	Low	6	17	1.13	0.29–4.63	1.43	0.34–6.25
Ile-Val or Val-Val	Moderate	7	14	1.60	0.42–6.51	2.27	0.56–9.79
Ile-Ile	Moderate	16	16	3.20	0.99–11.73	4.14**	1.17–16.54
Ile-Val or Val-Val	High	6	13	1.48	0.36–6.21	1.86	0.44–8.16
Ile-Ile	High	21	19	3.54**	1.14–12.53	5.52***	1.64–21.25

OR, odds ratio; CI, confidence interval; AOR, adjusted OR; GST, glutathione S-transferase.

*Adjusted by multiple logistic regression for age, sex, incense burning, and cockroaches seen at home.

***P* < 0.05.

****P* < 0.01.

Table 4. ORs for asthma with GSTP1-105 genetic polymorphism in moderate and high pollution districts

GSTP1-105 polymorphism	District air pollution level	Asthmatic children (<i>n</i>)	Controls (<i>n</i>)	Crude OR	95% CI	AOR*	95% CI
Ile-Val or Val-Val	Moderate	7	14	1.00		1.00	
Ile-Ile	Moderate	16	16	2.00	0.65–6.52	1.54	0.41–5.97
Ile-Val or Val-Val	High	6	13	1.00		1.00	
Ile-Ile	High	21	19	2.39	0.78–8.00	3.79**	1.01–17.08

OR, odds ratio; CI, confidence interval; AOR, adjusted OR; GST, glutathione S-transferase.

*Adjusted by multiple logistic regression for age, sex, incense burning, and cockroaches seen at home.

***P* < 0.05.

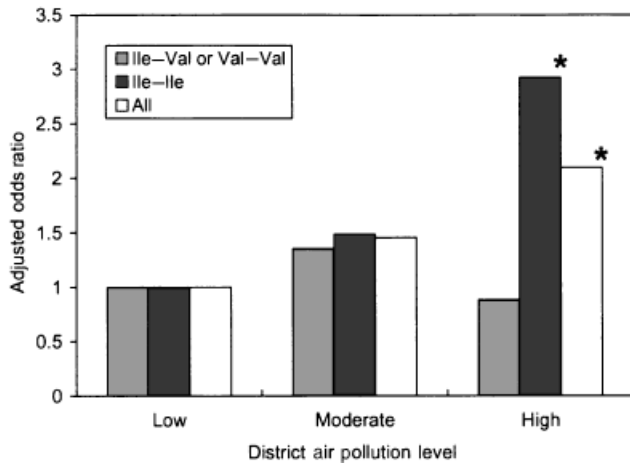


Fig. 2. Adjusted odds ratios for childhood asthma associated with different air pollution levels, with adjustment for age, sex, incense burning, and cockroaches seen at home. Associations were stratified by GSTP1-105 genotypes. The asterisks indicate statistical significance (* $P < 0.05$).

Because of the 100% DNA extraction rate, we could analyse the relationships between questionnaire-defined childhood asthma and outdoor air pollution by GSTP1-105 genotype. The interaction between the homozygous variant Ile-105 genotype and air pollution on the increased risk of asthma showed a clear dose-response relationship (Fig. 2). Compared with low air pollution, the AOR was 1.50 (95% CI: 0.74–3.05) for moderate air pollution and 2.92 (95% CI: 1.44–6.01) for high air pollution. However, outdoor air pollution showed no increased risk for childhood asthma in individuals with any Val-105 allele.

Discussion

We examined the relationships between the genotypic distribution of the GSTP1 polymorphism, outdoor air pollution, and childhood asthma using 61 schoolchildren with asthma and 95 controls, all selected from 2853 fourth- to ninth-grade schoolchildren from three districts in southern Taiwan. All participants in this study were homozygous at the Ala-114 locus. Although there was a marginally significant association between the frequency of homozygosity at the Ile-105 locus and childhood asthma when air pollution was not considered, after adjusting for confounding factors, our results suggested a significant interaction between air pollution and the GSTP1-105 genotype. Compared with participants carrying any Val-105 allele, those who were homozygous for Ile-105 had a significantly increased risk of asthma in high air pollution districts. In low or moderate air pollution districts, however, children carrying two Ile-105 alleles did not have the same increased risk.

Age, sex, ethnic factors, smoking habits, viral infections, and indoor environmental factors are believed to contribute to the occurrence of childhood asthma [23, 30]. We minimized interference from these confounders by recruiting lifelong non-smokers of similar age and approximately evenly divided by sex. All the recruited children had the same ethnic origin and none had had a viral infection during the 2 weeks before

the study. Although the associations did not reach statistical significance, the two most prevalent indoor causes of asthma in Taiwan, cockroaches and incense burning at home, showed positive effects on the incidence of childhood asthma (Table 2). In our preliminary study [31], we suggested that sensitization to cockroach allergens, rather than to cat or dog dander, was associated with lower pulmonary functions. Incense burning at home, which contributes a substantial amount of indoor particulate matter in most homes in Taiwan, was also a risk factor of increased cord blood IgE and fetal airway symptoms in our birth cohort study [32]. In fact, exposure to tobacco smoke and other housing factors, like water damage and visible mould on walls at home, were also considered in our survey. These factors showed negative effects to the occurrence of childhood asthma (data not shown) because they were easily changed upon human behaviours through selection mechanisms, especially in cross-sectional study. Therefore, we only controlled cockroaches and incense burning at home as potential confounders in the analyses.

BHR may be modulated by ROS levels, possibly through their ability to regulate eicosanoid production by stimulating the release of arachidonic acid [33]. The GST genes are candidates for a role in BHR because the enzymes they encode modulate ROS levels [8, 9]. Our results support the hypothesis that individual ability to detoxify ROS and their products, determined by polymorphism in GSTP1, contributes to the development of childhood asthma. This view is also confirmed by studies showing that individuals with reduced antioxidant capacity are at increased risk of allergic asthma, and that decreased intake of antioxidants is associated with the expression of asthma-related phenotypes [7, 34].

There is much evidence that long-term exposure to ambient air pollution increases the prevalence of childhood asthma. In two recent studies, the risk of severe asthmatic symptoms in Los Angeles was associated with exposure of 1.4 ppb 8 h nitrogen dioxide (NO_2) [14], and the risk of physician-diagnosed asthma in the Netherlands was associated with traffic-related air pollution measured as NO_2 concentration [15]. Another study of identical cross-sectional design in the Czech Republic and Poland [16] showed that the lifetime prevalence of physician-diagnosed asthma in schoolchildren was related to SO_2 . In our previous study in Taiwan [17], traffic-related air pollution, especially NO_x concentrations, was associated with prevalence of childhood asthma. The present study has shown that air pollution was not only a risk factor for childhood asthma, but also a modifiable cause of genetic susceptibility. However, in our study design, it was not possible to elaborate the kinds or amounts of air pollutants that had direct effects on our study population's airways.

Although individuals may vary in genetic susceptibility to outdoor air pollution, there are still limited epidemiological studies on the effects of air pollutants on GSTP1 activities. Compared with GSTP1 Ile-105, enzymes with Val-105 have a sevenfold greater catalytic efficiency for polycyclic aromatic hydrocarbon diol epoxides but a threefold lower efficiency for 1-chloro-2,4-dinitrobenzene [35]. In a human inhalation challenge study, Gilliland et al. [36] found that people with GSTP1 Ile-105 homozygotes showed larger IgE and

histamine increases in nasal lavage fluid after challenge with diesel exhaust particles and allergens. Constantin et al. [37] also suggested that people with higher GST activity were less sensitive to exposure to carcinogens, like the metabolites of SO₂ and NO₂. In the present study, we found the risk of asthma increased (AOR = 5.52; 95% CI: 1.64–21.25) in those who both live in high air pollution area and are Ile-105 homozygotes (Table 3), and there was a clear dose–response curve with outdoor air pollution levels (Fig. 2). Our results provide an important demonstration of a gene–environmental interaction. Because different polymorphic forms of the GSTP1 gene at position 105 have different effects on the detoxification ability of an individual, it is interesting to speculate that an air pollution-induced airway injury might be different in children carrying different polymorphic forms of this gene. Among the schoolchildren who lived in the high air pollution area, we found that children with asthma were more than 3.7 times as likely as controls to carry the homozygous Ile-105 genotype (Table 4). Additional research is necessary to prove that the elimination of environmental exposures among genetically susceptible individuals could reduce rates of childhood asthma.

The ecologic exposure assessment had many advantages in our study. The density of elementary and middle schools in Taiwan was very high, and almost all the surveyed children attended schools within 1 km of their homes. Monitoring stations located near the schools were also likely to be near the students' homes, and thus provided good indicators for both school and home exposure. Migrating from one district to another could lead to misclassification of exposure. However, errors in exposure assessment were likely to be random, which would reduce the magnitude of association, but would not introduce a positive bias in the associations. The exposure information obtained from air-monitoring stations was limited to the air pollutant criteria in 1994 and later years. Using the air pollution data from 1994 to 2001 in exposure assessment was rational in considering the latency period of asthma. Bias could be introduced if differential changes of air pollutants were found between communities, but it seems no certain situation in recent years (Fig. 1).

The GSTP1 gene resides on chromosome 11q13, which is a hot spot for asthma-related gene. The high-affinity IgE receptor (FcεR1β) gene also maps in this region and may be dependent on its association with childhood asthma [38]. It is possible that our results reflect linkage disequilibrium between GSTP1 alleles and the true candidate gene, located near chromosome 11q13 [39]. We analysed the most possible polymorphic change at amino acid 237 from glutamic acid to glycine (E237G) in our study population; however, no significant association was found. Therefore, our result in this study must not be biased since our hypothesis is that ROS elicits a key component of inflammation in the development of childhood asthma, and the role of the association between cross-linking of the receptor and cytokine/immunoglobulin production may be minimal.

Results from our laboratory showed that the frequency of GSTP1 Ile-105 allele seems to be higher in the Taiwanese population than in Caucasians. The frequency of GSTP1 Ile-105 allele in western countries ranged from 64.3% [12], 68.4% [36], to 72.3% [40] throughout Britain, America, and Northern Europe. In contrast, here we found that the frequency was

around 74.7% (controls) to 83.6% (asthmatic children) in the Taiwanese population. The high frequency of the Ile-105 allele in the Taiwanese population may make the OR of Ile-105 homozygote carriers for acquiring asthma lower than that of Caucasian populations. Nonetheless, our data did provide an alternative explanation for linkage of the chromosome 11q13 region to childhood asthma.

Questionnaires have been widely used to assess childhood asthma. We applied composite diagnostic criteria assuring diagnoses of asthma-positive (cases) and asthma-negative (controls), but this did not allow us to dissect which of the phenotypic characteristics were most strongly responsible for the observed gene by environmental interaction. The past history of physician-diagnosed asthma or dyspnea with wheezing was our major outcome measurement. Overestimation of the true prevalence of asthma might occur if these criteria were used in parental-reported questionnaire surveys. The lack of power because of our relatively small sample size was a major concern. We did not have genotyping data from all subjects, which made selection bias possible. However, there were no substantial differences between the demographic characteristics of those with genotypes and those without genotypes (data not shown), and DNA extraction rate was 100% in the recruited population, suggesting that any bias from selection mechanism or availability of DNA is likely to be very small.

In summary, our results showed that outdoor air pollution was a modifiable cause of childhood asthma in a genetically susceptible subpopulation. We also found a significant gene–environmental interaction between the GSTP1 genotype and air pollution. This stresses the importance of air pollutants that may lead to lung injury, and also the variation in detoxification ability of the GSTP1 genetic polymorphism that may be used as markers of individuals susceptible to inhalational insults. Our data suggest that childhood asthma is a complex disease associated with many genes, and interactions between genes and environmental factors.

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