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Glutathione *S*-transferase, incense burning and asthma in children

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ABSTRACT: Incense burning is a popular practice in many family homes and temples. However, little is known about the effects of indoor incense burning and genetic polymorphisms on asthma. This study evaluated the effects of indoor incense burning and glutathione *S*-transferase (GST) genetic polymorphisms on asthma and wheeze.

In 2007, 3,764 seventh-grade schoolchildren (mean±SD age 12.42±0.65 yrs) were evaluated using a standard questionnaire for information about respiratory symptoms and environmental exposures. Multiple logistic regressions were performed to assess the association between GST polymorphisms and incense burning frequency on asthma and wheeze, after adjusting for potential confounders.

The frequency of incense burning at home was associated with increased risk of current asthma (p=0.05), medication use (p=0.03) and exercise wheeze (p=0.001). GSTθ1 (*GSTT1*) null genotypes were associated with current asthma (OR 1.43, 95% CI 1.00–2.04) and medication use (OR 1.46, 95% CI 1.01–2.22). *GSTT1* showed a significant interactive effect with incense burning on current asthma, current wheeze and nocturnal wheeze. The frequency of incense burning was associated with increased risk of current asthma, medication use, lifetime wheeze, nocturnal wheeze and exercise wheeze in an exposure–response manner among children with *GSTT1* null genotype (p<0.05).

Incense burning is a risk factor for asthma and wheezing, especially in *GSTT1* genetically susceptible children.

KEYWORDS: Asthma, gene–environment interaction, *GSTT1*, incense burning

The prevalence of asthma in Asia differs from that in western countries [1, 2]. Culture, lifestyle and residential environment are all potential risk factors that contribute to such differences [3]. Incense burning and its effect on respiratory health is worth exploring because exposure to incense smoke may be comparable to exposure to tobacco smoke, as incense burning is a traditional and popular practice among many families and in temples [4, 5]. In addition to burning huge amounts of incense in temples during major Chinese or religious festivals, many believers also burn incense when they worship at home or in temples.

The physical characteristics of the incenses are similar. Typical Chinese incense is composed of 35% fragrance material, 33% bamboo stick, 21% herbal and wood powder by weight, and 11% adhesive powder [6]. The emitted smoke contains particulate matter (PM), diethylphthalate (DEP), gas products and many other organic compounds. Due to its slow and incomplete combustion, incense burning produces continuous smoke that generates toxic gases and chemical particles, such as polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, isoprene and benzene, that can easily accumulate inside houses with inadequate ventilation [6]. It is reported that PAHs and airborne PM from incense burning constitute potential health hazards, which are related to respiratory symptoms, asthma, elevated cord blood immunoglobulin (Ig)E levels, contact dermatitis and cancer [7, 8]. Therefore, exploring the health effects of exposure to incense smoke in children is warranted.

The glutathione *S*-transferase (GST) superfamily includes a number of susceptibility genes. Several members, including GSTμ1 (*GSTM1*), GSTθ1 (*GSTT1*) and GSTπ1 (*GSTP1*), are expressed in the respiratory tract and involved in asthma pathogenesis, including oxidant defences, xenobiotic metabolism and detoxification of hydroperoxides [9].

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The *GSTT1* null genotype has been shown to be significantly associated with atopy [10]. Among *GSTT1*-deficient children, *in utero* exposure to smoke is also reportedly associated with significant decrements in lung function [11]. Furthermore, SAADAT and ANSARI-LARI [12] reported that both *GSTT1* and *GSTM1* null genotypes lack protection against asthma development in adults with a positive history of smoking. Evidence suggests that variants in the *GSTM1* and *GSTP1* loci may contribute to the occurrence of childhood asthma and increase susceptibility to adverse effects of air pollution [13]. Asthmatic children with *GSTM1* null and $GSTP1^{Val/Val}$ genotypes also appear more susceptible to developing respiratory symptoms related to ozone exposure [14]. However, whether or not polymorphism of the GST genotypes can modify the effects of incense smoke on asthma requires investigation. Thus, the purpose of this study was to evaluate the association between indoor incense burning and GST polymorphisms in children.

METHODS

Study population

Between September and October 2007, 5,082 middle-school children were recruited from public schools in 14 Taiwan Children Health Study communities. 3,764 seventh-grade school-aged children completed the standard questionnaire. Each student took home an informed consent form and a standard questionnaire to be answered by parents with additional questions concerning the effect of incense burning on asthma symptoms [15]. Questions in the questionnaire included basic demography, residential environmental factors (*e.g.* environmental tobacco smoke (ETS), pets and cockroaches at home, dampness of the house, fungus on the house walls, air cleaners, air conditioners, dehumidifiers, and carpets at home) and family history of atopic diseases. All of the selected subjects were of the same ethnic origin. The institutional review board (National Taiwan University Hospital Research Ethics Committee, Taipei, Taiwan; study number 200902042R) approved the study protocol, which is as previously described [15].

Health effects assessment

The parents' questionnaire responses were used to categorise children's asthmatic and wheezing status as previously described [15]. Children were considered as having lifetime asthma if the answer to the question ''Has a doctor ever diagnosed this child as having asthma?'' was ''yes''. Current asthma was defined as physician-diagnosed asthma with any asthma-related symptoms or illnesses in the previous 12 months. Medication use was defined as use of any inhaled, oral or intravenous medication in the previous 12 months. Lifetime wheeze was determined by a positive response to the question ''Has your child ever had wheeze or whistling in the chest at any time in the past when he/she did not have a cold or the flu?'' Those who reported attacks in the previous 12 months were identified as having current wheeze. Exercise wheeze was determined by a positive response to the question, ''Has your child ever had wheeze or shortness of breath triggered by exercise?''

Incense exposure

Since children were exposed to incense smoke mostly in their homes, incense smoke dosage was determined from the question ''How often is incense burned in the household when the child is in the room during the past 12 months?'' The frequency of incense burning was divided into three categories: never, less than daily (two times per month or during major festivals) and daily (all day long, every day, and morning and night every day).

Analysis of genetic polymorphisms

Cotton swabs containing oral mucosa were collected and stored at -80°C before analysis. Genomic DNA was isolated using the phenol/chloroform extraction method [16]. Genetic polymorphisms of the *GSTM1* and *GSTT1* genes were identified by PCR, while *GSTP1* polymorphisms were detected by the PCR–restriction fragment length polymorphism method, as previously described [16]. The laboratory staff was blinded to each subject's clinical status. Genotype assignments were based on two consistent experimental results. About 15% of the randomly selected samples were sequenced and all were consistent with the initial genotyping results.

Statistical analysis

Multiple logistic regression models adjusted for major covariates were made to examine the effects of incense burning with GST genotypes on asthma and wheezing. To further assess gene–environment interaction, the combined association of incense burning and GST genotypes was examined by stratifying into four groups: no incense exposure with *GSTT1* or *GSTM1* present, no incense exposure with *GSTT1* or *GSTM1* null, incense exposure with *GSTT1* or *GSTM1* present, and incense exposure with *GSTT1* or *GSTM1* null. With regard to *GSTP1*, the genotypes were also stratified into four groups: no incense exposure with $GSTP1^{Ile/Ile}$, no incense exposure with $GSTP1^{Ile/Val}$ or $GSTP1^{Val/Val}$, incense exposure with $GSTP1^{Ile/Ile}$, and incense exposure with $GSTP1^{Ile/Val}$ or $GSTP1^{Val/Val}$. Bonferroni correction was used to address the problem of multiple comparisons.

Gene–environment interaction was tested by adding a product term in the regression model. For categorical variables with more than two categories, the gene–environment interaction was evaluated using the likelihood ratio test, comparing the model with indicator variables for the cross-classified variables with a reduced model containing indicator variables for the main effects only. Within genotype categories, a one degree of freedom trend test was used to evaluate the possible exposure–response relationship across categories of the incense burning variables.

Odds ratios and 95% confidence intervals were adjusted for important potential confounders in all analyses. Selection of confounders that were included in the model was based on *a priori* consideration and standard statistical procedure of a 10% change in point estimates. Subjects with missing covariate information were included in the model using missing indicators. All hypothesis testing was two-sided at the significance level of 0.05 and performed using the SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the 3,764 children with complete questionnaire and genotyping data enrolled in this study, 26 were excluded due to active smoking. Of the 3,738 participants included in the final analysis, 63.0% had residential incense smoke exposure. Table 1 provides the demographic characteristics of the study

TABLE 1 Selected characteristics of participants in the Taiwan Children Health Study

	With genotyping	All eligible participants
Subjects n	3764	5082
Demographic information		
Males	1848 (49.1)	2464 (48.5)
Age yrs	12.26±0.50	12.42±0.65
Parental education# yrs		
⩽12	2319 (62)	3201 (63.4)
13–15	740 (19.8)	964 (19.1)
⩾16	679 (18.2)	884 (17.5)
Gestational age#		
⩽37	335 (9.1)	461 (9.3)
>37	3342 (90.9)	4496 (90.7)
Parental asthma#	114 (3.1)	140 (2.9)
Parental atopy#	957 (26.4)	1263 (25.8)
Family income# TWD		
⩽400000	1249 (35.8)	1777 (37.7)
400001–800000	1408 (40.4)	1853 (39.3)
⩾810000	828 (23.8)	1080 (22.9)
Active smoking#	26 (0.7)	37 (0.7)
Maternal smoking during pregnancy#	146 (3.9)	198 (3.9)
ETS at home#	1774 (47.4)	2471 (48.9)
Incense burning at home#		
Never	1326 (37.0)	1724 (35.8)
Less than daily	1021 (28.5)	1398 (29.0)
Daily	1237 (34.5)	1699 (35.2)
Respiratory outcomes#		
Asthma		
Lifetime asthma	292 (7.8)	375 (7.4)
Current asthma	114 (3.1)	140 (2.9)
Medication use	96 (2.6)	123 (2.4)
Wheeze		
Lifetime wheeze	449 (12.0)	586 (11.6)
Current wheeze	150 (4.0)	186 (3.7)
Nocturnal wheeze	82 (2.2)	109 (2.2)
Exercise wheeze	206 (5.5)	276 (5.5)

Data are presented as n (%) or mean±SD, unless otherwise stated. TWD: New Taiwan dollar; ETS: environmental tobacco smoke. #: number of subjects does not sum to total because of missing data.

population. The characteristics were almost identical between those with and without genotyping. The overall allele frequencies were 48.1% null *GSTT1*, 56.9% null *GSTM1* and 65.4% *GSTP1* Val105Ile polymorphisms.

The results showed that incense burning at home was associated with increased risk of current asthma (OR 1.36, 95% CI 1.01–2.00; p=0.05), medication use (OR 1.52, 95% CI 1.02–2.42; p=0.03) and exercise wheeze (OR 1.64, 95% CI 1.18–2.28; p=0.001) (table 2). The *GSTT1* null genotypes were associated with current asthma (OR 1.43, 95% CI 1.00–2.04) and medication use (OR 1.46, 95% CI 1.01–2.22). The *GSTT1* null genotype had a positive relationship with wheeze that did not reach statistical significance (table 2). Both the *GSTM1* null and *GSTP1*$^{Ile/Ile}$ genotypes were not significantly associated with asthma or wheeze.

As only the *GSTT1* null genotype was associated with asthma, the association between incense burning and *GSTT1* genotype on asthma and wheeze was further examined. In a mutually adjusted model of potential confounders, the *GSTT1* null genotype showed a significant interaction with incense burning on current asthma, current wheeze and nocturnal wheeze ($p<0.05$) (table 3). However, analyses of *GSTM1* and *GSTP1* did not show significant interactive effects (online supplementary tables). Incense burning and *GSTT1* had a synergistic effect on current asthma, with OR 1.40 (95% CI 0.76–2.57) for no incense exposure with *GSTT1* null genotype, OR 1.36 (95% CI 0.77–2.40) for incense exposure with *GSTT1* present genotype and OR 1.92 (95% CI 1.11–3.32) for incense exposure with *GSTT1* null genotype (table 3).

After stratification by *GSTT1* genotype, the frequency of incense burning was associated with increased risk of current asthma (p=0.04), medication use (p=0.02), lifetime wheeze (p=0.04), nocturnal wheeze (p=0.003) and exercise wheeze (p=0.01) in an exposure–response manner for children with the *GSTT1* null genotype (table 4). However, there were no significant relationships between incense burning and *GSTM1* and *GSTP1* genotypes on asthma and wheeze (online supplementary tables).

DISCUSSION

This study is a contribution to the literature on the potential associations between genetic polymorphisms, incense burning and paediatric asthma. The combination effects of *GSTT1* polymorphisms and incense smoke exposure on the risk of childhood asthma have not been previously studied. Asthma and wheezing are positively associated with the frequency of incense burning. In addition, children carrying the *GSTT1* null genotype are most susceptible to the adverse effects of incense smoke.

The factors examined (age, sex, parental education, family income, parental history of atopy, gestational age, residence, maternal smoking, pre- and post-natal ETS exposure, pets and cockroaches at home, dampness of the house, fungus on the house walls, and carpets at home) may all confound the results. As such, they are all considered as potential confounders in this survey and those who had a 10% change in point estimates in the statistical procedures have been adjusted. Those with smoking habits have also been eliminated as study subjects to avoid the confounding effect of active smoking. In addition, the subjects were all Han Chinese, with the premise that they are rather homogeneous. Therefore, the confounding effect of ethnicity was lessened.

As the frequencies of incense burning are associated with increased risk of current asthma and medication use, instead of lifetime asthma, it is suspected that incense burning may play a more important role in exacerbating asthmatic symptoms among *GSTT1* null children. Consistent with a previous epidemiological study, AL-RAWAS *et al.* [17] also report that incense burning is a common trigger of worsening of wheezing among asthmatic children, but is not associated with the prevalence of asthma. Furthermore, YANG *et al.* [18] have also discovered that incense burning and mosquito repellant

TABLE 2 Effects of incense burning at home and glutathione *S*-transferase genotype on subcategories of asthma and wheeze

	Asthma			Wheeze			
	Lifetime asthma	Current asthma	Medication use	Lifetime wheeze	Current wheeze	Nocturnal wheeze	Exercise wheeze
Incense burning	1.05 (0.80–1.36)	1.36 (1.01–2.00)	1.52 (1.02–2.42)	1.10 (0.88–1.37)	1.07 (0.75–1.53)	1.31 (0.8–2.14)	1.64 (1.18–2.28)
Frequency of incense burning[#]							
Never	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Less than daily	0.76 (0.54–1.07)	1.13 (0.70–1.83)	1.29 (0.72–2.30)	0.92 (0.70–1.21)	1.00 (0.64–1.56)	1.07 (0.58–2.01)	1.62 (1.09–2.41)
Daily	1.22 (0.90–1.64)	1.52 (0.99–2.34)	1.75 (1.03–2.96)	1.21 (0.94–1.55)	1.12 (0.74–1.70)	1.68 (0.97–2.93)	1.88 (1.29–2.74)
p-value for trend	0.20	0.05	0.03	0.13	0.60	0.06	0.001
***GSTT1* null**	1.21 (0.95–1.56)	1.43 (1.00–2.04)	1.46 (1.01–2.22)	1.17 (0.95–1.44)	1.14 (0.81–1.60)	1.14 (0.72–1.79)	1.09 (0.81–1.47)
***GSTM1* null**	0.87 (0.68–1.12)	0.89 (0.63–1.27)	0.84 (0.55–1.27)	0.98 (0.80–1.21)	0.91 (0.65–1.27)	0.73 (0.47–1.16)	0.90 (0.67–1.21)
***GSTP1*$^{Ile/Ile}$**	1.18 (0.60–2.32)	0.89 (0.37–2.11)	0.72 (0.28–1.85)	0.96 (0.57–1.61)	0.83 (0.37–1.86)	1.21 (0.37–4)	1.76 (0.7–4.41)

Data are presented as odds ratios with 95% confidence intervals. Models are adjusted for age, sex, parental education, parental asthma, parental atopy, gestational age, city, maternal smoking during pregnancy and environmental tobacco smoke at home. [#]: average days of incense burning at home per month.

burning are significantly associated with acute cough symptoms in primary school children.

In contrast, KOO *et al.* [19] have found that there is no association between incense burning and respiratory symptoms among primary school children and their nonsmoking mothers in Hong Kong. The age of the study population and the constituents of incense from different countries are all be possible contributors to discrepancies in findings.

The biological mechanisms by which the toxic effects of incense burning results in asthma are not well understood. It was reported that burning incense emitted CO, CO_2, NO_2, SO_2, DEP and PM. Exposures to NO_2 and SO_2 can lead to

TABLE 3 Association between incense burning at home and *GSTT1* genotype on subcategories of asthma and wheeze

	No incense		With incense	
	***GSTT1* present**	***GSTT1* null**	***GSTT1* present**	***GSTT1* null**
Univariate model				
Asthma				
Lifetime asthma	1.00	1.12 (0.76–1.65)	0.87 (0.61–1.25)	1.11 (0.79–1.58)
Current asthma[#]	1.00	1.37 (0.77–2.45)	1.09 (0.63–1.87)	1.56 (0.93–2.63)
Medication use	1.00	1.39 (0.67–2.88)	1.25 (0.64–2.44)	1.87 (0.99–3.55)
Wheeze				
Lifetime wheeze	1.00	1.08 (0.78–1.48)	0.87 (0.65–1.17)	1.12 (0.84–1.49)
Current wheeze	1.00	0.96 (0.57–1.62)	0.79 (0.49–1.27)	1.03 (0.65–1.63)
Nocturnal wheeze[#]	1.00	0.62 (0.28–1.36)	0.84 (0.45–1.58)	1.19 (0.65–2.16)
Exercise wheeze	1.00	1.27 (0.75–2.14)	1.67 (1.06–2.62)	1.68 (1.07–2.66)
Mutually adjusted model[¶]				
Asthma				
Lifetime asthma	1.00	1.14 (0.76–1.70)	0.99 (0.68–1.44)	1.25 (0.87–1.81)
Current asthma[#]	1.00	1.40 (0.76–2.57)	1.36 (0.77–2.40)	1.92 (1.11–3.32)
Medication use	1.00	1.32 (0.62–2.79)	1.42 (0.71–2.85)	2.12 (1.09–4.12)
Wheeze				
Lifetime wheeze	1.00	1.08 (0.77–1.50)	1.02 (0.75–1.39)	1.27 (0.94–1.72)
Current wheeze[#]	1.00	1.02 (0.59–1.76)	0.96 (0.58–1.60)	1.20 (0.73–1.97)
Nocturnal wheeze[#]	1.00	0.65 (0.29–1.45)	0.87 (0.44–1.70)	1.30 (0.69–2.46)
Exercise wheeze	1.00	1.33 (0.77–2.28)	1.89 (1.17–3.05)	1.91 (1.18–3.09)

Data are presented as odds ratios with 95% confidence intervals. [#]: significant interaction; [¶]: models are adjusted for age, sex, parental education, parental asthma, parental atopy, gestational age, city, maternal smoking during pregnancy and environmental tobacco smoke at home.

TABLE 4 Association of frequency of incense burning at home on subcategories of asthma and wheeze by *GSTT1* genotype

	GSTT1 present	*GSTT1* null
Subjects n	1937	1801
Frequency of incense burning#		
Asthma		
Lifetime asthma		
Never	1.00	1.00
Less than daily	0.74 (0.45–1.21)	0.74 (0.45–1.22)
Daily	1.04 (0.66–1.63)	1.42 (0.94–2.13)
p-value for trend¶	0.87	0.09
Current asthma		
Never	1.00	1.00
Less than daily	1.22 (0.60–2.50)	1.05 (0.54–2.05)
Daily	1.37 (0.69–2.75)	1.78 (1.01–3.16)
p-value for trend¶	0.37	0.04
Medication use		
Never	1.00	1.00
Less than daily	1.22 (0.51–2.92)	1.35 (0.60–3.00)
Daily	1.37 (0.59–3.18)	2.26 (1.11–4.57)
p-value for trend¶	0.47	0.02
Wheeze		
Lifetime wheeze		
Never	1.00	1.00
Less than daily	0.96 (0.65–1.41)	0.85 (0.57–1.26)
Daily	1.02 (0.70–1.49)	1.40 (1.00–1.98)
p-value for trend¶	0.91	0.04
Current wheeze		
Never	1.00	1.00
Less than daily	0.78 (0.40–1.50)	1.15 (0.62–2.14)
Daily	1.01 (0.55–1.87)	1.17 (0.65–2.11)
p-value for trend¶	0.97	0.60
Nocturnal wheeze		
Never	1.00	1.00
Less than daily	0.86 (0.38–1.96)	1.20 (0.45–3.23)
Daily	0.85 (0.37–1.95)	3.21 (1.41–7.30)
p-value for trend¶	0.69	0.003
Exercise wheeze		
Never	1.00	1.00
Less than daily	2.03 (1.16–3.54)	1.28 (0.71–2.31)
Daily	1.86 (1.06–3.28)	1.99 (1.18–3.35)
p-value for trend¶	0.04	0.01

Data are presented as odds ratios with 95% confidence intervals, unless otherwise stated. Models are adjusted for age, sex, parental education, parental asthma, parental atopy, gestational age, city, maternal smoking during pregnancy and environmental tobacco smoke at home. #: average days of incense burning at home per month; ¶: calculated by stratification with *GSTT1* genotype.

respiratory illness, reduced pulmonary function and alterations in the lung's defence system [20]. Incense burning also produces volatile organic compounds, such as benzene, toluene, isoprene, xylenes, aldehydes and PAHs [6]. These compounds may lead to nose and throat irritation, asthma exacerbation and cancer.

Aside from irritated respiratory tracts, LIN *et al.* [21] also report that incense burning may cause elevated cord blood IgE. It is plausible that PM stimulates dendritic cells and T-cells to produce T-helper cell type 2 cytokines and activate pro-inflammatory genes in a process mediated by free radical and oxidative stress mechanisms [22]. Since exposure to lead can stimulate IgE production, it is speculated that lead emitted from incense burning first attaches to PM, subsequently transferring to fetal blood and modulating the fetal immune system with IgE production [21, 23]. Furthermore, incense smoke also causes morphological changes of pneumocytes and infiltrates of neutrophils in rat alveoli [24]. Activation of inflammatory cells may lead to amplification of various mediators, ending in inflammatory changes and airway remodelling [25].

Frequency of incense burning is associated with increased risk of asthma in an exposure–response manner in this study. After stratification by *GSTT1* genotypes, the effect of incense burning remains only in the *GSTT1* null genotype. *GSTT1* also shows significant synergistic interaction with incense burning on current asthma, with OR 1.40 (95% CI 0.76–2.57) for no incense exposure with *GSTT1* null genotype, OR 1.36 (95% CI 0.77–2.40) for incense exposure with *GSTT1* present genotype and OR 1.92 (95% CI 1.11–3.32) for incense exposure with *GSTT1* null genotype. Previously, however, variants in the *GSTM1* and *GSTP1* loci reportedly contribute to the occurrence of childhood asthma, thereby increasing susceptibility to adverse effects of tobacco smoke [10, 14]. The differences in genotype findings compared to other studies can be due to ethnic compositions and various distributions of genotype frequencies. There is a higher frequency of *GSTT1* null genotypes in this study population than in Caucasians (48.1 *versus* 25.0%) [26]. Such inconsistent results may underscore the inherent weakness of single-gene analyses for the study of gene–environment interactions for a multigene disease like asthma. Since different polymorphisms of the *GSTT1* gene have different effects on the detoxification ability of an individual, an incense smoke-induced airway injury is different in children carrying different *GSTT1* polymorphisms. The statistically suggested interactions in this study add to the plausibility of a biological interaction between the *GSTT1* enzyme and incense smoke in the detoxification process of reactive metabolic intermediates and reactive oxygen species [27, 28]. Furthermore, CORNELIS *et al.* [27] report that consumption of cruciferous vegetables is associated with lower risk of myocardial infarction only among those with a functional *GSTT1* allele, which suggests that *GSTT1* carriers can protect against oxidative stress or DNA damage [27, 29].

Consistent with previous studies, the *GSTT1* null genotype is associated with increased risk of asthma [11, 30]. A possible explanation for this is that GST enzymes, largely expressed in human lung cells, act as detoxifying enzymes and serve as markers of putative oxidative stress [31]. Furthermore, altered antioxidant defences, lipid peroxidation and anti-inflammatory pathways are important in asthma pathogenesis [32]. It can be speculated that asthma caused by incense smoke partially contributes to DNA damage, which can occur from the lack of detoxification of reactive smoke metabolites by the *GSTT1* enzyme [26, 33]. However, evidence for an association between GSTs and asthma is inconsistent. The findings of MINELLI *et al.* [34]

do not support a substantial role of GST genes alone in the development of asthma. Future large-scale studies about interactions of GST genes with environmental oxidative exposures and other genes involved in antioxidant pathways among genetically susceptible individuals are necessary.

This study has some potential limitations that may influence the interpretation of the results. First, the degree of ventilation of the room where incense was burned is not known. The amount of incense smoke inhaled will be biased, because incense exposure has been calculated based on the questionnaire. There is also no good quantitative biomarker for incense burning presently. Exposure assessments from the questionnaire are regarded as appropriate surrogate measurements of incense smoke exposure, which may be subject to misclassification bias. Furthermore, the study subjects are likely to be exposed to other sources of incense aside from their own houses. If incense smoke exposure from temples or other places are included in the analyses, the current amount of incense smoke used will most likely double or triple. Misclassification bias will then shift the results toward the null. Another possible limitation is recall bias in respiratory outcomes. The recall of asthma status was assessed in a subset of the study population and found that the concordance of parental reports of asthma and medical records' documentation of asthma was good.

One of the strengths of this study is its inclusion of a large and sociodemographically diverse population of children in Taiwan. Unbiased observations of the association between genetic polymorphisms and outcomes are expected. In addition, the association between incense burning has been investigated with many respiratory outcomes. To our knowledge, the interaction of genetic polymorphisms and incense burning on respiratory manifestations has not been previously reported. The results reported here suggest that genotyping for *GSTT1* polymorphism, a simple and inexpensive assay, may be a suitable biomarker for identifying genetically susceptible children.

In conclusion, household incense exposure has adverse effects on children carrying the *GSTT1* null genotypes. These findings not only help us to understand the aetiology of asthma but also guide potential control measures in the future. The diverse ability to detoxify incense smoke depends on variations of *GSTT1* polymorphisms. As incense smoke is a complex mixture of chemicals, and since other metabolic genes may be involved, additional long-term research is needed to explore the relative role of other genes in determining genetic susceptibility to adverse respiratory outcomes.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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