Glutathione S-Transferase, incense burning, and asthma in children

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Short title: GSTT1, incense burning, and childhood asthma

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ABSTRACT

Incense burning is a popular practice in many families 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, 3764 seventh-grade schoolchildren 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). Glutathione S-transferase theta-1 (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 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.

KEY WORDS: *Asthma, gene-environmental interaction, GSTT1, incense burning*

INTRODUCTION

The prevalence of asthma in Asia differs from that of western countries [1,2]. Culture, lifestyle, and residential environment are all potential risk factors that contribute to such difference [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 because incense burning is a traditional and popular practice among many families and 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 IgE 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) super-family includes a number of susceptibility genes. Several members, including glutathione S-transferase mu 1 (GSTM1), glutathione S-transferase theta-1 (GSTT1), and glutathione S-transferase pi 1 (GSTP1), are expressed in the respiratory tract and involved in asthma pathogenesis, including oxidant defenses, xenobiotic metabolism, and detoxification of hydroperoxides [9]. 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 et al. report that both GSTT1 and GSTM1 null genotypes lack protection against asthma development in adults with positive history of smoking [12]. 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, 5082 middle-school children were recruited from public schools in 14 Taiwan Children Health Study (TCHS) communities. The focus population was on 3764 seventh-grade school-aged children who 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 wall, air cleaner, air conditioner, dehumidifier, 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; Number: 200902042R) approved the study protocol, which is as previously described [15].

Health effects assessment

The parents' questionnaire responses were used to categorize 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 a yes. Current asthma was defined as physician-diagnosed asthma with any asthma-related symptoms or illnesses in the past 12 months. Medication use was defined as use of any inhaled, oral, or intravenous medication in the past 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 past 12 months were identified as 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 3 categories: never, less than daily (2 times per month or in 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 recognized by polymerase chain reaction (PCR) while the GSTP1 was detected by PCR-restriction fragment length polymorphisms (PCR- RFLP) 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-environmental interaction, the combined association of incense burning and GST genotypes was examined by stratifying into four groups: no incense GSTT1 or GSTM1 present, no incense GSTT1 or GSTM1 null, with incense GSTT1 or GSTM1 present, and with incense GSTT1 or GSTM1 null. As regards GSTP1, the genotypes were also stratified into four groups: no incense GSTP1 Ile-Ile, no incense GSTP1 Ile-Val or Val-Val, incense GSTP1 Ile-Ile, and incense GSTP1 Ile-Val or Val-Val. Bonferroni correction was used to address the problem of multiple comparisons.

Gene-environmental interaction was tested by adding a product term in the regression model. For categorical variables with more than two categories, the gene-environmental 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 category, a one degree of freedom trend test was used to evaluate the possible exposure-response relationship across categories of the incense burning variables.

Odds ratio (OR) and 95% confidence interval (CI) 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 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).

RESULTS

Of the 3764 children with complete questionnaire and genotyping data enrolled in this study, 26 were excluded due to active smoking. Of the 3738 participants included in the final analysis, 63.0% had residential incense smoke exposure. Table 1 provided the demographic characteristics of the study population. The characteristics were almost identical between those with and without genotyping. The overall allele frequencies were 48.1% null GSTT1 polymorphisms, 56.9% null GSTM1 polymorphisms, and 65.4% Ile GSTP1-105 polymorphisms.

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, but did not reach statistical significance (Table 2). Both the GSTM1 null and the homozygous GSTP1 Ile-105 were not significantly associated with asthma and wheeze.

Since 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 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 (Supplement 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 GSTT1 null genotype, OR=1.36 (95% CI=0.77-2.40) for incense exposure GSTT1 present genotype, and OR=1.92 (95% CI=1.11-3.32) for incense exposure 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 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 literature on the potential association among genetic polymorphisms, incense burning, and pediatric 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, gender, 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 wall, and carpets at home - may all confound the results. As such, they are all considered as potential confounders in this survey and those who made 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 is less.

Since 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 epidemiologic study, Al-Rawas et al. 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 [18]. Furthermore, Yang et al. have also discovered that incense burning and mosquito repellant burning are significantly associated with acute cough symptoms in primary school children [19].

In contrast, Koo et al. have found that there is no association between incense burning and respiratory symptoms among primary school children and their non-smoking mothers in Hong Kong [20]. 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 molecules emitted from burning incense include CO, CO2, NO2, SO2, DEP, and PMs. Exposures to NO2 and SO2 can lead to respiratory illness, reduced pulmonary function, and alterations in the lung's defense system [21]. Incense burning also produces volatile organic compounds (VOCs) like 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. also report that incense burning may cause elevated cord blood IgE [22]. It is plausible that PMs stimulate dendritic cells and T cells to produce Th2 cytokines and activate pro-inflammatory genes in a process mediated by free radical and oxidative stress mechanisms [23]. Since exposure to lead can stimulate IgE production, it is speculated that lead emitted from incense burning first attaches to PMs, subsequently transferring to fetal blood and modulating the fetal immune system with IgE production [22,24]. Furthermore, incense smoke also causes morphologic changes of pneumocytes and infiltrates of neutrophils in rat alveoli [25]. Activation of inflammatory cells may lead to amplification of various mediators, ending in inflammatory changes and airway remodeling [26].

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. The 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 GSTT1 null genotype, OR=1.36 (95%

CI=0.77-2.40) for incense exposure GSTT1 present genotype, and OR=1.92 (95% CI=1.11-3.32) for incense exposure 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 genotype in this study population than in Caucasians (48.1% vs. 25.0%) [27]. Such inconsistent results may underscore the inherent weakness of single-gene analyses for the study of gene-environment interactions for a multi-gene 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 (ROS) [28,29]. Furthermore, Cornelis et al. 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 [28,30].

Consistent with previous studies, the GSTT1 null genotype is associated with increased risk of asthma [11,31]. 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 [32]. Furthermore, altered anti-oxidant defenses, lipid peroxidation, and anti-inflammation pathways are important in asthma pathogenesis [33]. 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 [27,34].

However, evidence for an association between GSTs and asthma is inconsistent. The findings of Minelli et al. do not support a substantial role of GST genes alone in the development of asthma [35]. 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 as regards 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 socio-demographically 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. By current knowledge, the interaction of genetic polymorphisms and incense burning on respiratory manifestations has not been previously reported. The results here suggest that genotyping for the 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 understand the etiology of asthma but also guide potential control measures in the future. The diverse detoxification ability of incense smoke depends on variations of GSTT1 polymorphisms. Since incense smoke is a complex mixture of chemicals and other metabolic genes may be involved, additional long-term research is warranted to explore the relative role of other genes in determining genetic susceptibility to adverse respiratory outcomes.

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

	With genotyping (n=3764)		All eligible participants (n=5082)	
	n	%	n	%
Demographic information				
Boy	1848	49.1	2464	48.5
Age, yr (Mean ± SD)	12.26 ± 0.50		12.42 ± 0.65	
Parental education, yr†				
<=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*†				
<=400,000	1249	35.8	1777	37.7
400,001~800,000	1408	40.4	1853	39.3
>=810,000	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

*New Taiwan dollars per year ($1 USD = $ 33 New Taiwan dollar).
†Number of subjects does not add up to total N because of missing data.

TABLE 2 Effects of incense burning at home and GST genotype on sub-categories of asthma and wheeze

	Asthma					
	Lifetime asthma		Current asthma		Medication use	
	OR	95%CI	OR	95%CI	OR	95%CI
Incense burning	1.05	(0.80,1.36)	1.36	(1.01,2.00)	1.52	(1.02,2.42)
Frequency of incense burning*						
Never	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)
Daily	1.22	(0.90,1.64)	1.52	(0.99,2.34)	1.75	(1.03,2.96)
p value for trend	0.20		0.05		0.03	
GSTT1 Null	1.21	(0.95,1.56)	1.43	(1.00,2.04)	1.46	(1.01,2.22)
GSTM1 Null	0.87	(0.68,1.12)	0.89	(0.63,1.27)	0.84	(0.55,1.27)
GSTP1 Ile/Ile	1.18	(0.60,2.32)	0.89	(0.37,2.11)	0.72	(0.28,1.85)

	Wheeze							
	Lifetime wheeze		Current wheeze		Nocturnal wheeze		Exercise wheeze	
	OR	95%CI	OR	95%CI	OR	95%CI	OR	95%CI
Incense burning	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	
Less than daily	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	1.21	(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	0.13		0.60		0.06		0.001	
GSTT1 Null	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	0.98	(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	0.96	(0.57,1.61)	0.83	(0.37,1.86)	1.21	(0.37,4)	1.76	(0.7,4.41)

Models are adjusted for age, gender, parental education, parental asthma, parental atopy, gestational age, city, maternal smoking during pregnancy, and ETS at home

*Average days of incense burning at home per month

TABLE 3 Association between incense burning at home and GSTT1 genotype on sub-categories of asthma and wheeze

	No incense GSTT1 present	No incense GSTT1 null		With incense GSTT1 present		With incense GSTT1 null	
		OR	95%CI	OR	95%CI	OR	95%CI
	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)

*Models are adjusted for age, gender, parental education, parental asthma, parental atopy, gestational age, city, maternal smoking during pregnancy, and ETS at

home
†Signification of interaction

TABLE 4 Association of frequency of incense burning at home on sub-categories of asthma and wheeze by GSTT1 genotype

	GSTT1 present (n=1937)		GSTT1 null (n=1801)	
Frequency of incense burning*	OR	95%CI	OR	95%CI
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	

Models are adjusted for age, gender, parental education, parental asthma, parental atopy, gestational age, city, maternal smoking during pregnancy, and ETS at home

*Average days of incense burning at home per month

†Calculated by stratification with GSTT1 genotype