

The interaction of glutathione S-transferase M1-null variants with tobacco smoke exposure and the development of childhood asthma

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Clinical & Experimental Allergy

Summary

Background The glutathione S-transferase M1 (GSTM1)-null variant is a common copy number variant associated with adverse pulmonary outcomes, including asthma and airflow obstruction, with evidence of important gene-by-environment interactions with exposures to oxidative stress.

Objective To explore the joint interactive effects of GSTM1 copy number and tobacco smoke exposure on the development of asthma and asthma-related phenotypes in a family-based cohort of childhood asthmatics.

Methods We performed quantitative PCR-based genotyping for GSTM1 copy number in children of self-reported white ancestry with mild to moderate asthma in the Childhood Asthma Management Program. Questionnaire data regarding intrauterine (IUS) and post-natal, longitudinal smoke exposure were available. We performed both family-based and population-based tests of association for the interaction between GSTM1 copy number and tobacco smoke exposure with asthma and asthma-related phenotypes.

Results Associations of GSTM1-null variants with asthma ($P = 0.03$), younger age of asthma symptom onset ($P = 0.03$), and greater airflow obstruction (reduced forced expiratory volume in 1 s / forced vital capacity, $P = 0.01$) were observed among the 50 children (10% of the cohort) with exposure to IUS. In contrast, no associations were observed between GSTM1-null variants and asthma-related phenotypes among children without IUS exposure. Presence of at least one copy of GSTM1 conferred protection.

Conclusion These findings support an important gene-by-environment interaction between two common factors: increased risk of asthma and asthma-related phenotypes conferred by GSTM1-null homozygosity in children is restricted to those with a history of IUS exposure.

Keywords asthma, copy number variation (CNV), gene-by-environment, GSTM1, intrauterine smoke exposure, tobacco smoke

Submitted 11 May 2009; revised 29 July 2009; accepted 10 August 2009

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Cite this as: A. Rogers, C. Brasch-Andersen, I. Ionita-Laza, A. Murphy, S. Sharma, B. Klanderman and B. Raby, *Clinical & Experimental Allergy*, 2009 (39) 1721–1729.

Introduction

Glutathione S-transferase M1 (GSTM1) facilitates the scavenging of oxygen free radicals by catalysing the reaction between glutathione (GSH) and organic hydroperoxides. GSTM1 is located on chromosome 1p13.3, a region known to be copy-number variable for a common large-scale deletion that spans GSTM1. Among Caucasians the GSTM1-null allele has a frequency of 70%, meaning that the approximately one-half of Caucasian

individuals are GSTM1-null (homozygous for the deletion) and lack functional GSTM1.

GSTM1-null status has been implicated in poor outcomes in several auto-immune diseases, including systemic lupus erythematosus and rheumatoid arthritis. It has been clearly established as an important gene in chronic obstructive pulmonary disease (COPD). Its role in defense against oxidative stress also makes it a logical asthma candidate, and in fact, GSTM1 has been extensively studied as an asthma candidate gene, with more than 20 publications to date.

Yet the data for GSTM1 are conflicting. While several studies show increased risk of asthma or lung function decrements in all GSTM1-null subjects [1–8], others have identified such outcomes only in those subjects exposed to increased oxidative stress (tobacco smoke [9–11], or ozone [12–14], for example). Still others have found that absence of GSTM1 does not affect pulmonary outcomes, but rather modulates the effects of other glutathione *S*-transferases (such as GSTP1) [15, 16]. Functional data are limited, but also have not supported a role of GSTM1 genotype in asthma pathogenesis. Ercan *et al.* [17] showed that asthmatics had lower levels of reduced glutathione and higher levels of malondialdehyde, consistent with higher levels of oxidative stress, but found no difference based on GSTM1 presence or absence. Similarly, Mak *et al.* [18] found that GST activity was higher in asthmatics but, again, did not vary with GSTM1 genotype.

Herein we report results from our efforts to characterize the joint interactive effects of GSTM1 copy number and smoke exposure on the development of asthma and related phenotypes in a large cohort of asthmatic children who were participating in the Childhood Asthma Management Program (CAMP), for which detailed smoke exposure data and longitudinal measures of pulmonary outcomes are available.

Methods

Population

The CAMP is a multi-centre North American clinical trial designed to investigate the long-term effects of inhaled anti-inflammatory medications in children with mild to moderate asthma. A diagnosis of asthma was based on methacholine hyperreactivity [PC₂₀ (provocative concentration causing a 20% decline in forced expiratory volume in 1 s (FEV₁)) no > 12.5 mg/mL] and one or more of the following criteria for at least 6 months in the year before recruitment: (1) asthma symptoms at least two times per week, (2) at least two uses per week of an inhaled bronchodilator, or (3) daily asthma medication. Lung function measurements were obtained 13 times over the course of the 4-year follow-up (at time 0, 2, 4, and 8 months and then three times yearly throughout the trial). IgE and methacholine PC₂₀ were assessed twice and five times, respectively. Exposure to environmental tobacco smoke (ETS), including intrauterine smoke (IUS, any history of maternal smoking during pregnancy) and post-natal ETS (history of smoke exposure in the household by a parent or primary caregiver between birth and enrollment), was assessed at baseline via questionnaire response by a child's caregiver. ETS exposure was also assessed at each follow-up visit. Protocols for collection of baseline phenotypic data have been described in detail elsewhere [19, 20]. Of the 1041 children enrolled in the original

clinical trial, 968 children and 1518 of their parents contributed DNA samples to the CAMP Genetics Ancillary Study.

The Institutional Review Boards of the Brigham and Women's Hospital and of the other CAMP study centres approved this study. Informed assent and consent were obtained from the study participants and their parents to collect DNA for genetic studies.

Genotyping methods

Two thousand and three individuals (including 708 CAMP probands) were genotyped for GSTM1 copy number by real-time PCR with the Applied Biosystems (Foster City, CA, USA) Taqman copy number assay on a 7900HT instrument as described previously [21], using off-the-shelf GSTM1 (FAM labeled) and RNaseP (VIC labeled, serving as endogenous two-copy reference) probes. Because of the small number of samples from non-white ethnic groups and concerns regarding genetic heterogeneity, we restricted this analysis to the self-described white subjects and their available parents ($n = 1630$, including 517 white probands). Each sample was run in quadruplicate, with the ΔC_t averaged over the four runs. Continuous copy number values are assigned according to the $\Delta\Delta C_t$ method by ABI SDS software. Ambiguous and failed calls were re-run. Continuous copy number variation (CNV) measures were binned into discrete CNV genotype calls (i.e. zero copies 1–3). Ninety subjects were genotyped twice to assess reproducibility.

Statistical analysis

Population-based analysis was performed using R v.2.4.1 and SAS v.9.1 (Cary, NC, USA). We tested for CNV association with lung function phenotypes including post-bronchodilator FEV₁, forced vital capacity (FVC), the ratio of FEV₁/FVC, and log-transformed total serum IgE level and methacholine PC₂₀. We tested for association with CNV both at baseline (adjusting for age, gender, and height as appropriate) and longitudinally using repeated measures [additionally adjusted for ongoing tobacco smoke exposure and CAMP treatment assignment (inhaled budesonide, nedocromil, or placebo)]. For the longitudinal analysis, we constructed a linear mixed-effect model assuming an exponential decay in covariance using the PROC MIXED procedure in SAS. We tested for GSTM1 interaction with IUS and ETS exposure variables. Data were stratified for further analysis when significant interactions were noted.

Family-based testing was performed with PBAT v.5.3.0 (Bozeman, MT, USA) for asthma affection status and all variables assessed in the population-based analyses above, using both baseline and longitudinal measures

[22, 23]. Haploview was used to obtain counts of allele transmission [24].

Our primary analyses were run with a dominant model for GSTM1 (consistent with previous association results). We secondarily tested for association using an additive genetic model. Power calculations were performed using QUANTO version 1.2 [25, 26].

Results

Genotyping

Over 98% of subjects were successfully genotyped by quantitative PCR. Reproducibility was excellent, as 89 of the 90 subjects had the same binned genotype call on repeat genotyping. Because of concerns for population stratification, we restricted analysis to the Caucasian individuals. As shown in Fig. 1, the continuous CNV estimates obtained were easily binned into discrete genotype calls. In total, 282 probands were GSTM1 null, 191 were hemizygotes (i.e. had one copy of GSTM1), and 38 were homozygous for GSTM1 (i.e. had two or more

functional copies of GSTM1). The null allele frequency was 71%, similar to the findings of other groups among Caucasian individuals.

Association of glutathione S-transferase M1 copy number with asthma phenotypes

Baseline characteristics of the 511 CAMP subjects are noted in Table 1. Among all subjects, we found no evidence of association between GSTM1-null status and asthma affection, spirometric measures of lung function (FEV₁, FVC, and FEV₁/FVC), total serum IgE levels, or airway hyperresponsiveness to methacholine (Table 2). Repeated measures analysis also showed no association with any of these phenotypes (all $P > 0.1$). Although GSTM1-null status was associated with younger age of onset of asthma in family-based testing ($P = 0.01$), similar associations were not observed in population-based testing.

In contrast, we found evidence for consistent gene-by-environment interactions between GSTM1 and smoke exposure in association with asthma and related outcomes. Stratified analysis revealed that GSTM1-null individuals with a history of IUS exposure had earlier age of onset of asthma, higher FVC, and a lower FEV₁/FVC (Table 2) than IUS-exposed individuals with at least one copy of GSTM1. Transmission of the GSTM1-null allele was associated with asthma affection status ($P = 0.03$) with a more than twofold increased ratio of transmitted to untransmitted alleles (T:U ratio of 23:10). The age of onset of asthma was 1.5 years lower among IUS-exposed subjects who were GSTM1-null than those with at least one copy

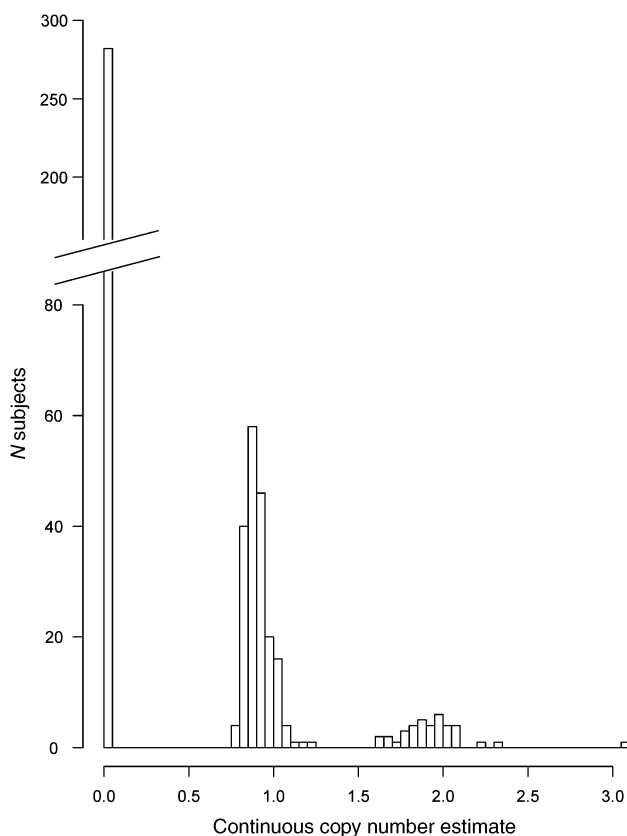


Fig. 1. Glutathione S-transferase M1 (GSTM1) genotyping among white probands, using Taqman qPCR. The 511 probands fall into several distinct bins, with 282 GSTM1 null, 191 with one copy, 37 with two copies and, one outlier subject with three copies of GSTM1, confirmed on repeat genotyping. Please note that Y-axis has a break for improved visibility of low-frequency copy number variation bins.

Table 1. Baseline characteristics of 511 white CAMP probands

Characteristic	Value
Age (years)	8.8 (2.1)
Male gender (%)	316 (62%)
Age of onset of asthma	3 (2.4)
FEV ₁ % predicted	103 (13)
FVC % predicted	107 (13)
FEV ₁ /FVC	85 (6)
Total IgE (ng/mL)	405 (159, 1086)
Methacholine PC ₂₀	1.1 (.5, 2.7)
Intrauterine smoke exposure (%)	50 (10%)
History of post-natal exposure to environmental tobacco smoke (%)	175 (34%)

All values shown are at baseline, off all controller medication for asthma. Mean (SD) or median (IQR) are shown as appropriate for normality of the data. Four hundred and eighty-nine of the 511 probands had complete trio data available for family-based testing; characteristics of this subset were similar.

CAMP, Childhood Asthma Management Program; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; IQR, inter-quartile range; PC₂₀, provocative concentration causing a 20% decline in FEV₁.

Table 2. GSTM1-null status is associated with multiple asthma phenotypes among the subset exposed to IUS

	All subjects		Smoke exposed only					
	GSTM1-null (n = 282)	GSTM1 present (n = 229)	Association P-value		GSTM1-null (n = 29)	GSTM1 present (n = 21)	Association P-value	
			Population-based	Family-based			Population-based	Family-based
Asthma	N/A	N/A	N/A	0.2	N/A	N/A	N/A	0.03*
FEV ₁ (%)	103.6	103.1	0.6	1	104.2	102.9	0.4	0.8
(predicted)								
FVC (%)	107.4	106.5	0.5	0.7	110.5	104.7	0.04 [†]	0.1
(predicted)								
FEV ₁ /FVC	85.4	85.7	0.7	0.7	83.8	86.9	0.03 [†]	0.01*
PC ₂₀ (mg/dL)	0.09	0.10	0.9	0.7	0.05	-0.04	0.7	0.3
IgE (IU/mL)	2.6	2.6	0.5	0.9	2.5	2.4	0.9	0.3
Age of Onset (years)	3.0	3.0	0.8	0.01*	2.5	4.1	0.04	0.03*

P-values represented here are for baseline association, using linear regression for population-based testing, and FBAT for family-based testing. Age of onset of asthma adjusted only for gender. IgE and LnPC₂₀ also adjusted for age. Lung function variables additionally adjusted for height and height squared.

*Transmission of the GSTM1-null allele was associated with asthma, earlier age of onset of asthma, and a lower FEV₁/FVC ratio.

[†]FEV₁/FVC and FVC were additionally significant in longitudinal analysis with $P = 0.02$ and 0.003 , respectively. Longitudinal analyses for FEV₁, PC₂₀, and IgE showed no association ($P > 0.05$).

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GSTM1, glutathione S-transferase M1; IUS, intrauterine smoke; PC₂₀, provocative concentration causing a 20% decline in FEV₁.

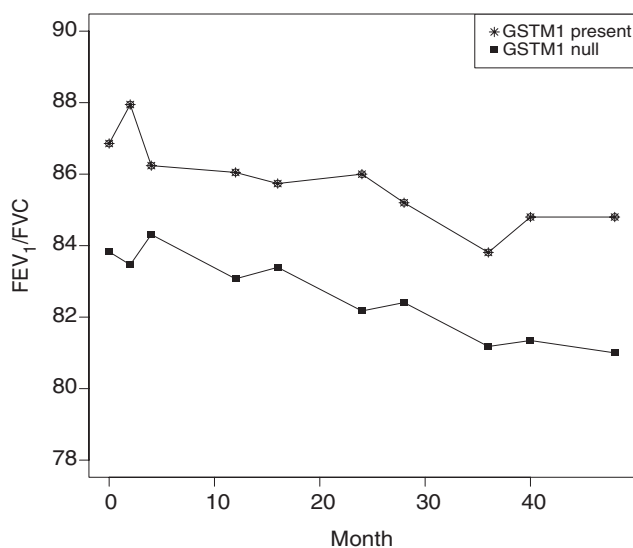


Fig. 2. Forced expiratory volume in 1 s (FEV₁)/forced vital capacity (FVC) reduction among glutathione S-transferase M1 (GSTM1) null, intrauterine smoke-exposed subjects persists over the 4-year-trial period. Mean FEV₁/FVC values over the 48-month trial are depicted.

of GSTM1 (mean age 2.5 vs. 4.1, $P < 0.05$ in both population and family-based testing). GSTM1-null status was also associated with lung function, with higher FVC and lower FEV₁/FVC. This was noted both at baseline (Table 2) and with repeated measures analysis over the 4-year trial ($P = 0.003$ and 0.02 , respectively). As shown in Fig. 2, among IUS-exposed GSTM1-null subjects, the mean

FEV₁/FVC remained approximately 3% lower throughout the follow-up period ($P = 0.02$). GSTM1 status was not associated with any of the asthma phenotypes among subjects not exposed to IUS (data not shown). Formal testing for interaction between GSTM1 genotype and smoke exposure in population-based models confirmed significant interaction terms for FEV₁/FVC and age-of-onset ($P < 0.05$).

Interaction between GSTM1 and post-natal ETS exposure was similar but of lower magnitude than the IUS interaction; GSTM1-null status is associated with lower baseline FEV₁/FVC, and younger age of onset ($P = 0.08$ and 0.05 in population-based testing, for example, among the 175 subjects with ETS exposure). IUS is highly correlated with ETS, however, with 46 of the 50 IUS-exposed subjects reporting ETS as well. When analysis is limited to the 129 ETS-exposed subjects without IUS exposure, we find no evidence of association between GSTM1 copy number and any of the variables tested.

One plausible explanation for association in the IUS-exposed subset is that the child's GSTM1 copy number is simply a surrogate for maternal GSTM1 copy number (i.e. smoking causes higher oxidative stress *in utero* among mothers who lack GSTM1, which leads to long-term effects on the child's risk for asthma and lung function). This was not the case in this cohort; the GSTM1 associations with asthma and lung function were noted only with the child's genotype, not the mother's. Testing outcomes

in an additive model (as opposed to dominant) did not reveal any additional associations.

Discussion

We demonstrate evidence for association of the GSTM1-null variant with asthma affection, age of asthma symptom onset, and pulmonary function among children with a history of passive smoke exposure. Although our results are compelling because of their consistency in both population- and family-based analysis (the latter rules out cryptic population stratification as a biasing confounder), our findings are best understood in the context of previously published studies. Table 3 summarizes all prior studies that assessed asthma affection status. Although statistically significant evidence of increased asthma risk among GSTM1-null carriers was observed in only seven of 20 populations previously studied, trends of similar association [i.e. odds ratio (OR) > 1] were observed in 12 of the 14 publications in which an OR was either reported or could be calculated from the published data. Given the large sample sizes typically required to demonstrate statistically significant modest genetic effects, the consistency in the direction of association across most of these studies suggests that the effects observed across populations are likely meaningful.

Although asthma is known to be highly heritable, with relative risk to siblings of an asthmatic of ~3, the role of environmental exposures in asthma pathogenesis is critical and is often underappreciated in genetic association studies. We note that, though many potential asthma genes are emerging from candidate gene and genome-wide association studies [27, 28], the vast majority of even the most promising asthma candidate genes have failed to show association in multiple other populations. One explanation for such disparate results in different populations is a failure to account for gene-by-environment interaction. Our data and others suggest that defining the importance of GSTM1 in asthma requires careful consideration of such interactions. It is certainly plausible that the cohorts that showed GSTM1 effects in all subjects simply had higher levels of exposure to ETS, ozone, or other sources of increased oxidative stress.

Our cohort, with 511 asthmatics and 489 complete trios, is among the largest asthmatic cohorts tested for GSTM1 association (see Table 3). The study was thus powered to appreciate clinically meaningful differences in the entire cohort (with 80% power to find a change in FEV₁ of 4%, or onset of asthma that was 7 months earlier, for example); adequate power makes our findings of no association between GSTM1 and various asthma phenotypes in the entire cohort more meaningful. In contrast to the findings in all subjects, we found strong evidence of association with asthma among subjects exposed to IUS (with 23 : 10 transmitted : untransmitted ratio of the GSTM1-null

allele). Similarly, GSTM1-null individuals who were exposed to IUS had a younger age of asthma onset and a lower FEV₁/FVC ratio than those with at least one copy of GSTM1; GSTM1 status did not influence these phenotypes in subjects without IUS exposure. We also note that four of the five studies that assessed GSTM1 in the context of high oxidative stress found significant association (Table 3); similarly, all studies that evaluated GSTM1 copy number and environmental effects on lung function were significant (Table 4). Thus, the literature strongly supports a role for environmental modulation of GSTM1 effects.

Our results are most similar to those of Gilliland et al. [9] who found that in a population-based cohort of 2590 children, associations of the GSTM1-null variant with active asthma, medication use, and earlier age of asthma onset were restricted entirely to children with a history of *in utero* smoke exposure. We note that early onset of asthma was assessed only in Gilliland's work and ours, and both studies identified significant GSTM1 effects in the context of IUS exposure. Given the putative mechanism of GSTM1 as a defense against oxidative stress, exposure to IUS or ETS early in life could logically result in an earlier age of asthma onset among those who lack GSTM1, suggesting that this phenotype may be of particular interest for studying genetic interactions with early-life exposures.

GSTM1-null status was associated with a lower FEV₁/FVC and higher FVC in IUS-exposed subjects in our cohort. These differences were present at baseline and sustained over the 4-year trial; in contrast, the slope of FEV₁/FVC and FVC did not change with GSTM1 status. These lung function findings are most consistent with dysanapsis, in which airway size is relatively smaller than lung parenchyma [29]. Nicotine exposure has been shown to stimulate lung branching morphogenesis and increase lung size in embryonic murine explants [30], while IUS exposure is associated with increased airway collagen deposition in rhesus monkeys [31] and with increased airway thickness in human infants [32]. While our results are consistent with the hypothesis that the anti-oxidant effects of GSTM1 prevent such changes in lung vs. airway growth *in utero*, this has not been previously examined.

A strength of this study is our use of quantitative PCR to genotype GSTM1 for zero, one, or two copies. Most previous publications genotyped only GSTM1 presence or absence, and therefore are unable to distinguish whether the associations observed are driven by GSTM1 hemizygotes or homozygotes. Our results demonstrate that a single copy of GSTM1 is sufficient to confer protective effects. Because of the relatively small number of two-copy individuals in the IUS-exposed cohort ($N = 4$), we are underpowered to make firm inferences regarding additional effects of a second copy of GSTM1 in asthma.

We note that our findings were significant in the IUS-exposed subjects ($n = 50$) but not significant in the much

Table 3. Literature review: effects of GSTM1 null on asthma affection status

Paper	N	Children vs. Adults	Race/Nation	Asthma definition	Main effect*	Gene by Environment Interaction?	Interaction with other GSTs?
Fryer et al. [33]	127/44	Adult	White	Wheeze, cough, dyspnoea, or chest tight+BDR 15%+atopy	OR 1.13 (CI 0.6, 2.3) [†]		
Ivaschenko et al. [4]	109/90	Mixed	Russian	Specialist-dx asthma+recurrent sx's+PF variability > 30%	OR 3.49 (1.9, 6.4)		Additive effects with GSTT1
Gilliland et al. [9]	2950 pop (451 asthma)	Children	Mixed	MD-dx asthma	-	IUS: early-onset asthma, wheezing, active asthma, asthma treatment, ER visits among GSTM1- & IUS+no ETS effects	
Tamer et al. [6]	101/103	Adult	Turkish	Clinical asthma hx+BDR reversible	OR 2.34 (1.3, 4.2)		Several GSTM1/GSTT1/GSTP1 interactions sig
Saadat et al. [5]	85/85	Adult	Iranian	2 of 3: typical sx's, +BDR > 15%, > 20% PF variability	OR 3.2 (1.7, 6.1)		GSTM1*GSTT1 are additive
Kabesch et al. [10]	3054 population (268 asthma)	Children	German white	MD-dx asthma or MD-dx 'recurrent spastic or asthmatic bronchitis'	OR 1.19 (0.9, 1.5) [†]	GSTM1-/ETS+higher asthma OR than GSTM1 +/ETS-	Interaction with GSTT1
Brasch-Andersen et al. [1]	160 families (296 asthmatics+ parents)	Mixed	Danish	Recurrent cough, wheeze, dysp+ methacholine PC ₂₀	TDT - for asthma (+ for asthma+ RAST in recessive model)		
Lee et al. [34]	86 families (156 asthma+ parents)	Children	Danish	Clinical asthma sx's & effect of std. asthma meds	TDT+ for asthma		
Holla et al. [8]	82/184	Children	Taiwan	MD-dx asthma	OR 1.37 (0.8, 2.4)		GSTP1 effects noted if GSTM1+
Romieu et al. [13]	306/331	Adult	Caucasian	Current MD-dx asthma+sx+anti-asthma meds	OR 1.18 (0.9, 1.6)		No interaction with GSTT1
Palmer et al. [11]	151	Children	Mexico City	Clinical sx & response to rx	OR of mod-severe asthma 2.05 (vs. mild, no control group)	Ozone. Ozone more difficulty breathing	Additive ozone interaction effects with GSTP1 val/val
	504 asthmatics	Children	White	MD-dx asthma	1.34 (0.9, 2)	ETS: younger subset higher OR of asthma & ETS. Age-dept changes in FEV ₁ , PEFV vary w/ETS	

Ercan et al. [17]	196 mild/116 mod-sev/vs. ctrls: 187 and 68	Children	White	Intermittent wheeze + 12% BDR or response to rx or +PC ₂₀	OR 1.05 (0.7, 1.5) [†]	Interaction with GSTP1
Salam et al. [35]	3124 pop (476 asthma)	Children	Hispanic and non-Hispanic white	MD-dx	OR 1.21 (0.98, 1.5)	Interaction between GSTP1 & EPHX1
Hanene et al. [2]	105/112	Children	Tunisia	NR	OR 2.35 (1.3, 4.3)	
Kamada et al. [15]	391 kids, 462 adult/639 ctrl	Children & adult	Japanese	MD-dx asthma	–	GSTP1 effects only in GSTM1 +
	115 kids/184 ctrl	Children	Japanese	MD-dx asthma	–	GSTP1 effects only in GSTM1 +
Mak et al. [18]	315/315	Adult	Chinese	Expert panel criteria + cough/wheeze + FEV ₁ varies +/- 15% New-onset, MD-dx asthma	OR 0.75 (0.5, 1.03) [†]	
Imboden et al. [36]	4426 pop; 144 develop asthma	Adult	Swiss, 'Predominantly Caucasian'		OR 0.93 (0.7, 1.3) [†]	No interaction with GSTP1
Islam et al. [3]	1610 CHS; 152 develop asthma	Children	Hispanic or non-Hispanic white	MD-dx asthma	+ HR of 1.6 for new onset asthma during adolescence	No interaction with GSTP1 effects
This study	511 white asthmatic probands in 489 trios	Children	White	MD-dx + methacholine PC ₂₀	– for asthma + Early age of onset ↓ FEV ₁ /FVC	

[†]Literature search included the HuGENavigator site for GSTM1 and asthma, supplemented with a PubMed search for the terms 'GSTM1 and Asthma'. Included here are English-language studies with a primary outcome diagnosis of asthma.

Bold indicates statistically significant.

*Main effects presented.

OR or HR for asthma calculated by authors if no asterisk present (adjusted results presented if performed).

[‡]OR presented was calculated based on data from paper, where possible.

– negative for asthma in all subjects; unable to calculate OR from data presented in publication.

ETS, environmental tobacco smoke; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GSTM1, glutathione S-transferase M1; HR, hazards ratio; IUS, intrauterine smoke; MD-dx, doctor diagnosed; Sx, symptoms; Rx, treatment; BDR, bronchodilator response to albuterol; OR, odds ratio; PC₂₀, provocative concentration causing a 20% decline in FEV₁; PEFR, peak expiratory flow rate; PF, peak flow; TDT, transmission disequilibrium test.

Table 4. GSTM1 effects on lung function in asthma

Paper	N	Race	Asthma diagnosis	Children vs. adults	Lung Function Effect of GSTM1 null	Gene by Environment interaction?	Interaction with other GSTs?
Gilliland et al. [7]	1940 pop (287 asthma)	Mixed	MD-dx asthma	Children	↓ FEV ₁ & FVC growth over 4y; esp. in asthmatics		GSTM1*GSTP1 effects additive
Carroll et al. [37]	224 families with 418 children	Caucasian	MD-dx asthma + wheeze in past year	Children	In probands		Additive GSTP1, GSTM1
Holla et al. [8]	675 (~50% asthma)/331	Caucasian	Current MD-dx asthma + sx + anti-asthma meds	Adult	↑ FEV ₁ , FVC in siblings ↓ FEV ₁ /FVC; do not report FEV ₁ or FVC		
Romicu et al. [14]	158 asthmatics	Mexico City	Sx and response to rx	Children	-	↓ FEF 25–75 with ↑ ozone exposure, esp. in more severe asthma	
Palmer et al. [11]	504 asthmatics	White	MD-dx asthma	Children	NR	Ages 3–12, no ETS; ↓ FEV ₁ Ages 13–21 + ETS; ↓ PEFR IUS exposed: ↑ FVC, ↓ FEV ₁ /FVC	
This study	511 white asthmatic probands in 489 trios		MD-dx + methacholine challenge	Children	-		

larger subset exposed to ETS ($n = 175$). Yet 46 of the 50 subjects exposed to IUS were also exposed to ETS later in life, and tended to have more ongoing smoke exposure throughout the 4-year follow-up period. Because of this high degree of correlation between IUS and ETS, we are unable to discern whether the observed associations with IUS reflect specific gene-by-environment interactions that are established primarily *in utero*, or whether IUS is simply a marker for more extensive and sustained ETS exposure later in life. We acknowledge that our findings were significant only in a small subset of subjects, with correspondingly marginal *P*-values; though this subgroup analysis was planned given the previous GSTM1 literature, confirmation of the importance of the interactions of GSTM1 and smoke exposure in additional asthma cohorts is necessary.

In summary, we found that lack of GSTM1 is associated with asthma, an early onset of asthma, and low FEV₁/FVC among asthmatic children exposed to IUS. One copy of GSTM1 appears to be sufficient to confer protective effects. Our findings support the growing literature on the importance of GSTM1 in asthmatics exposed to tobacco smoke.

Acknowledgements

We thank all subjects for their ongoing participation in this study. We acknowledge the CAMP investigators and research team, supported by the National Heart, Lung, and Blood Institute (NHLBI), for collection of CAMP Genetic Ancillary Study data.

The CAMP Genetics Ancillary Study is supported by the NHLBI, N01 HL093076, R01 HL086601, U01 HL065899, P01 HL083069, and T32 HL07427 from the National Institutes of Health and the NHLBI. All work on data from the CAMP Genetic Ancillary Study was conducted at the Channing Laboratory and the Brigham and Women's Hospital under appropriate CAMP policies and human subjects protections.

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