CHEST

Interaction of Glutathione S-Transferase M1, T1, and P1 Genes With Early Life Tobacco Smoke Exposure on Lung Function in Adolescents

Xin Dai, MPH; Shyamali C. Dharmage, PhD; Gayan Bowatte, PhD; Nilakshi T. Waidyatillake, PhD; Jennifer L. Perret, PhD; Jennie Hui, PhD; Bircan Erbas, PhD; Michael J. Abramson, PhD; Adrian J. Lowe, PhD; John A. Burgess, PhD; Cecilie Svanes, PhD; and Caroline J. Lodge, PhD

BACKGROUND: Glutathione S-transferase (GST) genes are involved in the management of oxidative stress in the lungs. We aimed to determine whether they modify the associations between early life smoke exposure and adverse lung health outcomes.

METHODS: The Melbourne Atopy Cohort study (a high-risk birth cohort) enrolled 620 children and followed them prospectively from birth. We recorded perinatal tobacco smoke exposure, asthma, and lung function at 12 (59%) and 18 years (66%) and genotyped for *GSTM1*, *GSTT1*, and *GSTP1* (69%).

RESULTS: GST genotypes were found to interact with tobacco smoke exposure on lung function outcomes (P interaction $\leq$ .05). Only among children with *GSTT1* null genotypes was exposure to mother's, father's, or parental tobacco smoke in early life associated with an increased risk of reductions in prebronchodilator (BD) FEV_1 and FVC at both 12 and 18 years. These associations were not seen in children with *GSTT1* present. Similarly, only among children with *GSTM1* null genotypes was exposure to father's or parental smoking associated with reductions in pre- and post-BD FEV_1 and FVC at 18 years. Only among children with Ile/Ile genotypes of *GSTP1* was exposure to mother's smoking associated with increased risk of reduced FEV_1 at 18 years, but this was not the case among children with Val/Val or Ile/Val genotypes.

CONCLUSIONS: Our study provides evidence of interaction between early tobacco smoke exposure and GST genotypes on lung function. Carriers of GST null mutations and *GSTP1* Ile/Ile alleles may be more susceptible when exposed to tobacco smoke in early life. These findings support stronger recommendations to protect all infants from tobacco smoke exposure.

TRIAL REGISTRY: Australian and New Zealand Clinical Trials Registry; No.: ACTRN12609000734268; URL: http://www.anzctr.org.au/ CHEST 2019; 155(1):94-102

KEY WORDS: epidemiology (pulmonary); gene polymorphism; lung function; pediatric asthma; tobacco smoke exposure

ABBREVIATIONS: BD = bronchodilator; GLI = Global Lung Function Initiative; GST = glutathione S-transferase; MACS = Melbourne Atopy Cohort Study

AFFILIATIONS: From the Allergy and Lung Health Unit (Ms Dai and Drs Dharmage, Bowatte, Waidyatillake, Perret, Lowe, Burgess, and Lodge), Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, VIC, Australia; National Institute of Fundamental Studies (Dr Bowatte), Kandy, Sri Lanka; the Centre for Food and Allergy Research (Drs Dharmage and Lowe), Murdoch Children's

Exposure to tobacco smoke in early life has been found to increase the risk of developing asthma and lung function deficits in adolescence and adulthood.[1-5] However, not all children exposed to tobacco smoke in early life develop asthma, which suggests differences in genetic susceptibility.

There is increasing interest in genetic polymorphisms involved in oxidative stress pathways. One of the major enzymatic regulators of oxidative stress in the body is through glutathione S-transferase (GST) enzymes encoded by GST genes. GST enzymes are involved in the detoxification of a variety of foreign chemicals, including several reactive tobacco metabolites and reactive oxygen species.[6-8] Three major GST enzymes are encoded by the *GSTM1*, *GSTT1*, and *GSTP1* genes. Deficiencies in the enzyme activity in *GSTM1* and *GSTT1* are typically caused by deletion of the genes (null genotypes), whereas the most studied *GSTP1* variant contains two polymorphisms: an A → G transition at codon 105, which leads to the ile105val amino acid substitution.[9,10] Substituting Ile^{105} for Val^{105} significantly lowers GST enzyme activity.[11]

However, a 2010 systematic review including 22 studies did not support a substantial direct role of GST genetic polymorphisms in the development of asthma. The authors instead suggested the possibility of a gene-environment interaction influencing the risks of asthma and impaired lung function.[12] However, there are a limited number of longitudinal studies focusing on gene-environment interactions and measuring outcomes at different time periods in older children and adolescents, especially concerning the effect on lung function.

We aimed to investigate the relationship between GST genes and early life tobacco smoke exposure and their interaction on asthma and lung function at 12 and 18 years of age in the Melbourne Atopy Cohort Study (MACS). We have previously found in this cohort that early life tobacco smoke exposure was associated with asthma and lung function impairment at 18 years in girls.[13] We now investigate the potential for GST gene (*GSM1*, *GSTT1*, and *GSTP1*) polymorphisms to modify these associations.

Materials and Methods

Study Population

Between 1990 and 1994, 620 children were recruited (while in utero) into the MACS (Fig 1). All children had at least one first degree family member with allergic disease. Details of the study have been published elsewhere.[14] Data on exposures and respiratory outcomes for these participants were collected from birth to 18 years of age. MACS initially began as a randomized controlled trial of infant formula on weaning,[15] but has since been followed as an observational cohort. The initial phases to the 12-year follow-up of MACS were approved by The Mercy Maternity Hospital Ethics Committee. The 18-year follow-up was approved by the University of Melbourne and the Royal Children's Hospital Ethics Committees (reference No. 28035).

Tobacco Smoke Exposure

Tobacco smoke exposure was collected at the initial interview from mothers (median, 4 days before birth of the child; interquartile range, 26 days prior to birth to 2 days after birth). In this study, mother's or father's smoking in early life was defined by response to the following questions at baseline: Is mother smoking now? and Is father smoking now? Parental smoking in early life was defined as either mother or father smoking or both. Participants completed written questionnaires at 18 years of age.

Asthma

Current asthma at 12 years of age was defined as positive responses by parents to the following question: Any episodes of asthma or treatment with breathing medication within the last 12 months?

Current asthma at 18 years of age was defined by positive responses by the participant to the following question: Have you had any episodes of asthma or used medication for asthma in the last year?

Lung Function

Lung function was measured by spirometry at 12 and 18 years of age, according to standard techniques described by the American Thoracic Society.[16,17] The detailed procedures at both time points have been described elsewhere.[13] SpiroCard spirometers (SpiroCard PC

Research Institute, Parkville, VIC, Australia; the PathWest Laboratory Medicine of West Australia (Dr Hui), Perth, WA, Australia; School of Population and Global Health and School of Pathology and Laboratory Medicine (Dr Hui), The University of Western Australia, WA, Australia; Busselton Population Medical Research Institute (Dr Hui), WA, Australia; the School of Public Health & Preventive Medicine (Dr Abramson), Monash University, Melbourne, VIC, Australia; the School of Psychology and Public Health (Dr Erbas), La Trobe University, Melbourne, VIC, Australia; Centre for International Health, University of Bergen, Bergen, Norway; and the Department of Occupational Medicine (Dr Svanes), Haukeland University Hospital, Bergen, Norway.

Part of this article has been presented in poster form at the 29th Annual Scientific Conference of the International Society of Environmental Epidemiology (ISEE 2017), September 27, 2017, Sydney, NSW, Australia.

FUNDING/SUPPORT: This study was funded by the National Health and Medical Research Council of Australia, VicHealth, The Asthma Foundation and Nestle Australia. X. D., S. C. D., G. B., J. L. P., A. J. L., and C. J. L. are funded by the National Health and Medical Research Council of Australia; and G. B. and J. L. P. were funded by the Centre for Air Quality & Health Research and Evaluation of Australia in 2016-2017, and are funded by the National Health and Medical Research Council of Australia in 2018.

CORRESPONDENCE TO: Shyamali C. Dharmage, PhD, Centre for Epidemiology and Biostatistics, School of Population and Global Health, Level 3 207 Bouverie St, University of Melbourne, Melbourne, VIC 3010, Australia; e-mail: s.dharmage@unimelb.edu.au

DOI: https://doi.org/10.1016/j.chest.2018.08.1079

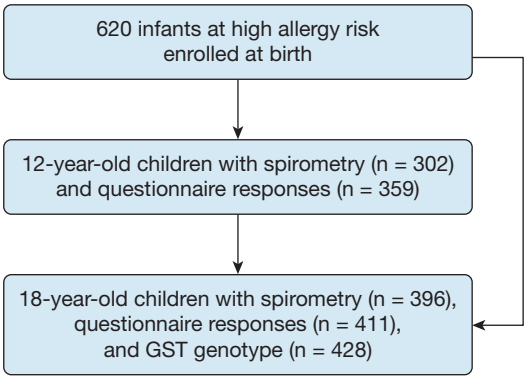

Figure 1 – *Flowchart of participants in the Melbourne Atopy Cohort Study. GST = glutathione S-transferase.*

Spirometer; QRS Diagnostic) were used at 12 years of age. The EasyOne Spirometer system (ndd Medical Technologies) was used for the 18-year follow-up, before and after the inhaled bronchodilator (BD) salbutamol 200 μg via a spacer. Tests were checked for acceptability and reproducibility by the respiratory scientist and one of the authors (C. J. L). FEV_1 and FVC and mid expiratory flow were measured. Both *z* scores (measures of SD from the mean predicted value) and % predicted values were calculated from the Global Lung Function Initiative (GLI) reference values for Caucasians.[18,19]

Genotyping

Blood or saliva was collected at the 18-year follow-up for GST genotyping.[14] Multiplex polymerase chain reaction techniques were used for measurement of the deletion of *GSTM1* and *GSTT1* genotypes. For all experiments, positive primers for beta-globin were included as a positive control. A customized GoldenGate Genotyping Assay (Illumina) was used to genotype the *GSTP1* (rs1695 A→G: ile105val) polymorphism. All assays were performed by scientists unaware of the clinical status of individual subjects. Genotype assignments were based on two consistent experimental results.

Other Variables

Baseline data also collected at the initial interview included parental age, parental education (used as a proxy for socioeconomic status), parental asthma, cooking facilities, and pets at home. Parental higher education was defined as either mother or father or both having a tertiary education at the time of study entry. Parental asthma was defined as either parent ever having a history of asthma. Adolescent smoking was defined by the following question at the 18-year follow-up: Have you smoked at least 100 cigarettes or an equal amount of cigars, pipes, or any other tobacco product?

Statistical Analyses

Multiple linear or logistic regression was used to assess the relationships between early life smoke exposure, GST genotype, adolescent lung function, and asthma outcomes. All ORs from regression models for asthma were adjusted for age, height, parental asthma at birth, and parental education. Results for associations with lung function parameters (FEV_1 and FVC) were expressed as *z* scores (SD and 95% CI) accounting for age, sex, height, and race using GLI reference values.[18,19] Results were also expressed in terms of % predicted values using GLI reference values.

We also considered cooking facilities, adolescent smoking, and pets at home as potential confounders. Variables were included in the multivariable model if they changed the effect on the associations (> 10% change in OR or coefficient).

For *GSTM1* and *GSTT1* genotypes, the reference group consisted of subjects with *GSTM1*/*GSTT1* wild-type alleles, and the exposed (risk) group was those with null genotypes. The referent group for *GSTP1* consisted of subjects having at least one Val allele (ie, Ile/Val, Val/Val). This was compared with the risk group, who were homozygous for Ile/Ile alleles. Strata-specific estimates were reported when an interaction *P* value ≤ .05 was observed. All analyses were conducted using Stata 14 (StataCorp LLC).

Results

MACS recruited slightly more girls (51.1%) than boys (48.9%). Only 38 (6.1%) were exposed to mother's smoking, and 112 (18.2%) were exposed to father's smoking. Overall, 127 (20.5%) were exposed to parental smoking. Spirometry was available for 366 (59%) and 411 (66.3%) at 12 and 18 years, respectively (Fig 1). *GSTM1*/*GSTT1* data were available for 428 participants, and *GSTP1* data were available for 429 participants (Table 1).

Main Effects of Early Life Smoke Exposure and GST Genes on Lung Function and Asthma

There was no association found between early life smoke exposure from parental smoking and lung function (e-Tables 1, 2, % predicted; Table 2, *z* scores) or asthma at age 12 and 18 years (e-Tables 3, 4). We did not find associations between GST null genotypes (*GSTM1* and *GSTT1*) for either lung function or asthma outcomes (e-Tables 5-8). *GSTP1* with Ile/Ile genotype was associated with an increased risk of reduced post-BD FVC at 18 years of age for children with Ile/Ile genotypes compared with those with Ile/Val or Val/Val (*z* score = −0.23; 95% CI, −0.42 to −0.03) (e-Table 6)

Interaction Analyses

Only among those with *GSTT*, *GSTM*, and *GSTP* risk genotypes was exposure to mother's, father's, or parental smoking associated with a reduction in lung function at 18 years of age.

Early Life Tobacco Smoke Exposure, GST Genes, and Lung Function Outcomes at 12 Years of Age: Because we found a significant interaction between *GSTT1* genotype and early life smoke exposure on lung function at 12 years of age (Table 2), we have presented these associations for different strata of GST gene exposure in Table 3.

TABLE 1] Selected Clinical Characteristics of the Study Cohort (N = 620)

Variables	Baseline	12-y Follow-Up	18-y Follow-Up
Sex			
Boy	317 (51.1)	190 (51.9)	208 (50.6)
Girl	303 (48.9)	176 (48.1)	203 (49.4)
Parental higher education			
Yes	417 (72.0)	268 (73.2)	309 (75.2)
No	162 (28.0)	98 (26.8)	102 (24.8)
Parental asthma			
Yes	356 (61.7)	228 (62.5)	253 (61.7)
No	221 (38.3)	137 (37.5)	157 (38.3)
Mother smoking			
Yes	38 (6.1)	16 (4.6)	16 (4.1)
No	582 (93.9)	333 (95.4)	370 (95.9)
Father smoking			
Yes	112 (18.2)	51 (14.6)	49 (12.7)
No	507 (81.8)	298 (85.4)	337 (87.3)
Parental smoking			
Yes	127 (20.5)	58 (16.6)	56 (14.5)
No	493 (79.5)	291 (83.4)	330 (85.5)
Adolescent smoking			
Yes	…	…	38 (9.3)
No	…	…	372 (90.7)
GSTM1			
Present	…	131 (44)	178 (41.6)
Null	…	167 (56)	250 (58.4)
GSTT1			
Present	…	249 (83.6)	353 (82.5)
Null	…	49 (16.4)	75 (17.5)
GSTP1			
Ile/Ile	…	132 (44.1)	184 (42.9)
Val/Ile and Val/Val	…	167 (55.9)	245 (57.1)

Values are No. (%).

Only among those with *GSTT1* null genotypes was exposure to mother's smoking associated with a significant reduction in lung function at 12 years of age (FEV_1: $z = -1.92$; 95% CI, −3.50 to −0.36; FVC: $z = -2.05$; 95% CI, −3.78 to −0.32). That is, *GSTT1* null children exposed to maternal smoking in early life had an increased risk of reduced FEV_1 and FVC by around 2 SDs from the predicted mean at 12 years of age when compared with those with null genotypes not exposed to maternal smoking. This association was not seen in children with *GSTT1* present genotypes. The interaction *P* value confirmed that the effect on lung function was not simply the cumulative effect from both exposures but the result of a synergistic (multiplicative) relationship between tobacco smoke exposure and *GSTT1* status on lung function.

Similarly, we found a significant interaction between *GSTT1* and parental smoking on lung function. Only among children with *GSTT1* null genotypes was exposure to parental smoking associated with an increased risk of reduced FEV_1 at 12 years of age (although the 95% CIs just crossed zero) compared with those with null genotypes but that were not exposed.

Similar analyses and findings for GLI-derived % predicted lung function parameter at 12 years of age are shown in e-Table 9.

TABLE 2] Main Associations Between Early Life Smoke Exposure and Lung Function at 12 and 18 Years of Age and Interaction *P* Values by GST Genes

Pre- or Post-BD	Smoking	Lung Function			Smoking × GST Interaction (*P* Value)		
			Unadjusted	Adjusted	*GSTM1*	*GSTT1*	*GSTP1*
12 y							
Pre-BD	Mother	FEV_1	0.34 (−0.23 to 0.91)	0.48 (−0.13 to 1.08)	.32	< .01[a]	.33
		FVC	1.26 (−0.51 to 0.71)	−0.13 (−0.64 to 0.39)	.79	< .01[a]	.82
	Father	FEV_1	−0.08 (−0.42 to 0.25)	0.26 (−0.38 to 0.90)	.53	.12	.08
		FVC	−0.10 (−0.46 to 0.25)	−0.10 (−0.47 to 0.27)	.73	.17	.27
	Parental	FEV_1	−0.09 (−0.41 to 0.22)	−0.07 (−0.40 to 0.26)	.51	.05	.29
		FVC	−0.16 (−0.49 to 0.18)	−0.11 (−0.46 to 0.23)	.66	.05	.50
18 y							
Pre-BD	Mother	FEV_1	−0.26 (−0.77 to 0.25)	−0.07 (−0.59 to 0.45)	.44	.03[a]	< .01[a]
		FVC	−0.21 (−0.72 to 0.29)	−0.13 (−0.64 to 0.39)	.43	.01[a]	.10
	Father	FEV_1	−0.20 (−0.52 to 0.11)	−0.11 (−0.42 to 0.21)	.15	.12	.60
		FVC	−0.03 (−0.34 to 0.28)	0.02 (−0.29 to 0.33)	.02[a]	.04[a]	.15
	Parental	FEV_1	−0.24 (−0.54 to 0.05)	−0.13 (−0.43 to 0.17)	.21	.09	.61
		FVC	−0.10 (−0.39 to 0.19)	−0.05 (−0.35 to 0.25)	.12	.01[a]	.30
Post-BD	Mother	FEV_1	−0.08 (−0.57 to 0.41)	0.07 (−0.42 to 0.57)	.44	044	.09
		FVC	0.03 (−0.47 to 0.53)	0.10 (−0.42 to 0.61)	.82	.32	.69
	Father	FEV_1	−0.20 (−0.52 to 0.11)	−0.02 (−0.32 to 0.28)	.03[a]	.68	.78
		FVC	−0.03 (−0.34 to 0.28)	0.05 (−0.26 to 0.36)	.02[a]	.89	.28
	Parental	FEV_1	−0.12 (−0.40 to 0.15)	−0.03 (−0.32 to 0.25)	.01[a]	.61	.55
		FVC	−0.03 (−0.31 to 0.25)	0.01 (−0.29 to 0.30)	.04[a]	.57	.41

Values are *z* score (SD, 95% CI) or *P* value. BD = bronchodilator; GST = glutathione S-transferase.
[a]Significant *P* values.

Early Life Tobacco Smoke Exposure, GST Genes, and lung Function Outcomes at 18 Years of Age: We also found some evidence for similar interactions at 18 years of age (Table 3). Only among children with *GSTM1* null genotypes was exposure to father's smoking associated with reductions in pre-BD FVC and post-BD FEV_1 and FVC at 18 years of age when compared with children with GST null not exposed to father's smoking in early life. Although the 95% CIs crossed 0, all the point estimates were reduced by around one-quarter of an SD. Again, the interaction *P* value confirmed that the effect on lung function was multiplicative when compared with either exposure alone. The positive interaction term for *GSTM1* may be partly related to the paradoxical relationship to early life smoke exposure in children with *GSTM1* present. Only among children with *GSTM1* present genotypes was exposure to father's tobacco smoke in early life associated with a significant increase in both pre and post-BD FVC when compared with children not smoke exposed. Significant interactions were also found for *GSTM1* null and father's smoking on pre-BD lung function and for *GSTM1* null and parental smoking on post-BD lung function. Interactions were also demonstrated for children with *GSTT1* null genotypes exposed to early life tobacco smoke on pre-BD lung function (Table 3). An isolated interaction was found for risk alleles of *GSTP1*. Only among children with *GSTP1* Ile/Ile genotypes was exposure to mother's smoking associated with an increased risk of reduced pre-BD FEV_1 compared with those with *GSTP1* Ile/Ile genotypes but not exposed. Similar analyses and findings for GLI-derived % predicted lung function parameters are shown in e-Table 9.

Early Life Tobacco Smoke Exposure, GST Genes, and Asthma/Wheeze at 12 and 18 Years of Age: There were no significant interactions found between early life smoking and GST polymorphisms on asthma or wheeze outcomes at 12 or 18 years of age (e-Tables 3-4).

We were unable to test adolescent smoking as an effect modifier because of the small numbers of adolescents who smoked (n = 38).

TABLE 3] Association Between Early Life Smoke Exposure and Lung Function at 12 and 18 Years of Age by GST Genotype

Pre- or Post-BD			Lung Function		*P* Interaction
12 y					
	Smoking		*GSTT1* Present	*GSTT1* Null	
Pre-BD	Mother	FEV_1	0.88 (0.81 to 1.68)	−1.92 (−3.50 to −0.36)	< .01
		FVC	0.66 (−0.20 to 1.53)	−2.05 (−3.78 to −0.32)	< .01
	Parental	FEV_1	−0.07 (−0.47 to 0.33)	−0.83(−1.75 to 0.09)	.05
		FVC	−0.07 (−0.50 to 0.37)	−0.93 (−1.93 to 0.06)	.05
18 y					
			GSTM1 Present	*GSTM1* Null	
Pre-BD	Father	FVC	0.69 (0.08 to 1.30)	−0.25 (−0.63 to 0.41)	.02
Post-BD	Father	FEV_1	0.47 (−0.15 to 1.10)	−0.26 (−0.62 to 0.09)	.03
		FVC	0.61 (0.01 to 1.21)	−0.22 (−0.60 to 0.16)	.02
	Parental	FEV_1	0.45 (−0.07 to 0.96)	−0.28 (−0.62 to 0.06)	.01
		FVC	0.40 (−0.10 to 0.91)	−0.24 (−0.61 to 0.12)	.04
			GSTT1 Present	*GSTT1* Null	
Pre-BD	Mother	FEV_1	0.30 (−0.35 to 0.95)	−0.76 (−2.00 to 0.48)	.03
		FVC	0.17 (−0.49 to 0.83)	−0.96 (−2.05 to 0.13)	.01
	Father	FVC	0.14 (−0.22 to 0.50)	−0.45 (−1.19 to 0.30)	.04
	Parental	FVC	0.12 (−0.22 to 0.46)	−0.71 (−1.43 to 0.01)	.01
			GSTP1 Ile/Val or Val/Val	*GSTP1* Ile/Ile	
Pre-BD	Mother	FEV_1	0.54 (−0.21 to 1.28)	−1.07 (−1.87 to −0.28)	< .01

Values are *z* score (SD, 95% CI) or as otherwise indicated. See Table 2 legend for expansion of abbreviations.

Discussion

In this study, we found evidence that individuals with *GSTM1* and *GSTT1* null genotypes were more susceptible to reduced FEV_1 and FVC at 18 years when exposed in early life to second-hand smoke. Similar associations were found for lung function at 12 years of age for early life smoke exposure and *GSTT1*. Although the trend was similar for asthma outcomes, interactions between the effects of GST genes and parental smoking were not significant.

The relevance of GST deficiency for lung function impairment and respiratory symptoms in children exposed to oxidative inhalants has been suggested by previous epidemiologic studies.[20-22] The Perth Infant Asthma Follow-up cohort investigated infant lung function at 12 months and found evidence for an interaction between smoke exposure in utero and *GSTT1* genes.[20] Kabesch et al[21] conducted a cross-sectional study of schoolchildren and found evidence suggesting increased susceptibility for asthma/wheeze in children with *GSTM1*/*GSTT1* null; however, the interactions were not significant. Our findings in a prospective birth cohort strengthen this growing body of evidence on this association up to late adolescence.

These findings are supported by strong biologic plausibility. Exposure to early life tobacco smoke, especially before 2 years of age, can damage the rapidly growing alveolar cells and stimulate inflammatory responses that may lead to development of impaired lung function and asthma in later life.[1,2,23] GST genes generate enzymes that detoxify and catalyze the conjugation of glutathione with oxidant substrates, including tobacco-derived substances. Through this mechanism, they may reduce inflammation and lung injury when exposed to oxidative stress.[24] Therefore, this may lead to impaired lung function when an individual has a GST gene deficiency (null genotype) or a reduction in enzymatic activity associated with genetic polymorphisms.

We found few differential effects on lung function for exposure to mother's and father's smoking in relation to *GSTM1* and *GSTT1*. However, effects related to mother's or father's smoking may differ because mothers tend to spend greater amounts of time with young children and mothers may also smoke during pregnancy. This

exposure in utero is thought to have different effects on the child than second-hand smoke exposure after birth. Although there was a suggestion that mothers smoking had a greater effect on lung function for children with *GSTT1* null genotypes and fathers smoking for *GSTM1* null genotypes, we were unable to draw definitive conclusions. The slight differences we observed may simply be because of lack of power associated with the small numbers of smoking mothers.

We did not find significant associations with asthma. Nicotine exposure in utero/early life may cause a global reduction in both FEV_1 and FVC rather than preferentially affecting FEV_1 as is the usual scenario for people with asthma.[25] Another possible reason could be that asthma is a dichotomous outcome, whereas lung function is a continuous outcome, leading to less power to detect an effect with asthma. A cross-sectional study investigating interactive effect between GST genes and passive smoking also found little effect on childhood asthma for *GSTM1*/*GSTT1* null groups although their sample size was $>$ 3,000.[21]

We found that among children with *GSTM1* present, those exposed to father's smoking in early life had an increased risk of improved lung function (FVC) compared with those unexposed. This result was inconsistent and unexpected. It is possible that this finding may be related to the small numbers of children exposed to both tobacco smoke and genotype. This contrary finding may also be because of compensation by other antioxidant genes or networks which are more successful at detoxifying substances other than tobacco smoke. There is evidence from two studies that a polymorphism (Ser187) of an alternative antioxidant enzyme, quinone oxidoreductase 1, provides a protective effect among *GSTM1* null subjects.[26,27] An alternative hypothesis is that tobacco smoke exposure in early life may induce upregulation of GST enzymes through DNA methylation changes. These improved oxidation defenses may then protect the lung from oxidative threats in the environment (other than tobacco smoke) that are also related to lung function. There is no conclusive support of this hypothesis in the literature.

Studies examining the *GSTP1* 105 variant reported that associations vary depending on the specific risk alleles of *GSTP1*.[28-30] Similar to our findings, some research exploring second-hand smoke has found no evidence of *GSTP1* interaction on asthma outcomes,[31-33] but studies investigating lung function were limited. We found no evidence of interaction for *GSTP1* genes with early life smoking and lung function impairment or asthma (interaction $P > .10$) One explanation may be because of the small numbers limiting our power to find associations. A further reason might be because the effect of *GSTP1* genes on allergy risk and lung function varies for different pollutants. For instance, enzymes encoded by Val genotypes have a sevenfold greater catalytic efficiency for polycyclic aromatic hydrocarbon diol epoxides but a threefold lower efficiency for 1-chloro-2,4-dinitrobenzene than those with Ile genotypes.[34,35] Careful measurement and definitions of pollutant exposures may be necessary before these relationships become clear.

Strengths and Limitations

An important strength of this study is the prospective birth cohort design. Early life smoking exposure was determined prospectively perinatally. Objective measurements of respiratory function were collected at 12 and 18 years of age. All these factors lessen any information bias, improving the validity of our findings.

Although our study collected early life tobacco smoke exposure data, these data were limited by the lack of information on the duration of smoking exposure, its intensity, and extent of exposure throughout life. In terms of personal smoking, very few of the adolescent participants had commenced smoking, making it unlikely for this to have materially impacted on the results. Moreover, MACS may have been underpowered to investigate the role of early life smoking exposure, particularly on asthma outcomes. We did not investigate other antioxidant genes/enzymes. There is a network of genes that interact with each other and form complex pathways that regulate oxidative stress and determine an individual's antioxidant capacity, including GST, quinone oxidoreductase 1, tumor necrosis factor, and Toll-like receptors.[36] Unfortunately, the limited size of this study precluded our ability to explore associations with other genes.

Additionally, as with all long-running cohorts, there was potential for attrition bias of our findings because data were obtained from only 66% of the children at 18 years of age. Furthermore, multiple testing may have generated spurious associations. We did not perform correction for multiple comparisons because our associations were based on pre-established hypotheses,

and the pattern of associations was consistent throughout the analyses. Finally, the high-risk nature of MACS may limit the generalizability of our results, and our findings should be validated in an independent cohort. The prevalence and degree of our observed lung function effects in our cohort would be higher than in a general population. However, it is likely that the interaction we found for high-risk children would also be present for high-risk individuals in a general population.

Conclusions

We found evidence that the impact of early life tobacco smoke exposure on reducing adolescent lung function was modified by GST gene polymorphisms, particularly *GSTT1*. These interactions may help to explain the inconsistent effects seen when either tobacco smoke exposure or GST genotypes were investigated alone for their effects on respiratory health.

Acknowledgments

Author contributions: At the 18-year follow-up, S. C. D., M. J. A., and A. J. L. were all involved with acquiring funding and/or establishing study directions and protocols. J. H. coordinated the blood collection and DNA genotyping at the 18-year follow-up. X. D. led the analysis and interpretation of the data with support from S. C. D., C. J. L., G. B., N. T. W., and J. L. P. X. D. wrote the initial draft of the manuscript, which was critically revised for important content by all the authors. All authors approved the final version of the article.

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following: M. J. A. has received investigator-initiated grants from Pfizer and Boehringer Ingelheim for unrelated research. None declared (X. D., S. C. D., G. B., N. T. W., J. L. P., J. H., B. E., A. J. L., J. A. B., C. S., C. J. L.).

Role of sponsors: The authors alone are responsible for the content and writing of the article.

Other contributions: We thank the children and families that participated in this research study and the investigators of the study up to the 12-year follow-up: David Hill, FRACP, Cliff Hosking, MD, John Thorburn, FRACP, and Christine Axelrad, BScN. We also thank the following investigators for help with the 18-year follow-up: Catherine Bennett, PhD, Lyle Gurrin, PhD, Rida Khalafzai, MD, Sharon Goldfeld, PhD, Chris Barton, PhD, Vijaya Sundararajan, FRACP, and Mathias Wjst, MD.

Additional information: The e-Tables can be found in the Supplemental Materials section of the online article.

References

1. Tager IB. The effects of second-hand and direct exposure to tobacco smoke on asthma and lung function in adolescence. *Paediatr Respir Rev*. 2008;9(1):29-37.
2. Gilliland FD, Berhane K, Li YF, Rappaport EB, Peters JM. Effects of early onset asthma and in utero exposure to maternal smoking on childhood lung function. *Am J Respir Crit Care Med*. 2003;167(6):917-924.
3. Alati R, Al Mamun A, O'Callaghan M, Najman JM, Williams GM. In utero and postnatal maternal smoking and asthma in adolescence. *Epidemiology*. 2006;17(2):138-144.
4. Wang XB, Wypij D, Gold DR, et al. A longitudinal-study of the effects of parental smoking on pulmonary-function in children 6-18 years. *Am J Respir Crit Care Med*. 1994;149(6):1420-1425.
5. Anderson WC III, Szefler SJ. New and future strategies to improve asthma control in children. *J Allergy Clin Immunol*. 2015;136(4):848-859.
6. Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology*. 2000;61(3):154-166.
7. Minelli C, Wei I, Sagoo G, Jarvis D, Shaheen S, Burney P. Interactive effects of antioxidant genes and air pollution on respiratory function and airway disease: a HuGE review. *Am J Epidemiol*. 2011;173(6):603-620.
8. Hanene C, Jihene L, Jamel A, Kamel H, Agnés H. Association of GST genes polymorphisms with asthma in tunisian children. *Mediators Inflamm*. 2007;2007(1):1-6.
9. Fryer AA, Bianco A, Hepple M, Jones PW, Strange RC, Spiteri MA. Polymorphism at the glutathione S-transferase GSTP1 locus. A new marker for bronchial hyperresponsiveness and asthma. *Am J Respir Crit Care Med*. 2000;161(5):1437-1442.
10. He JQ, Ruan J, Connett JE, Anthonisen NR, Pare PD, Sandford AJ. Antioxidant gene polymorphisms and susceptibility to a rapid decline in lung function in smokers. *Am J Respir Crit Care Med*. 2002;166(3):323-328.
11. Sun N, Sun X, Chen B, et al. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. *Cancer Chemother Pharmacol*. 2010;65(3):437-446.
12. Minelli C, Granell R, Newson R, et al. Glutathione-S-transferase genes and asthma phenotypes: a Human Genome Epidemiology (HuGE) systematic review and meta-analysis including unpublished data. *Int J Epidemiol*. 2010;39(2):539-562.
13. Dai X, Dharmage SC, Lowe AJ, et al. Early smoke exposure is associated with asthma and lung function deficits in adolescents. *J Asthma*. 2017;54(6):662-669.
14. Lowe AJ, Lodge CJ, Allen KJ, et al. Cohort Profile: Melbourne Atopy Cohort study (MACS). *Int J Epidemiol*. 2017;46(1):25-26.
15. Lowe AJ, Hosking CS, Bennett CM, et al. Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: a randomized controlled trial. *J Allergy Clin Immunol*. 2011;128(2):360-365.e4.
16. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med*. 1995;152(3):1107-1136.
17. Appleton SL, Adams RJ, Wilson DH, Taylor AW, Ruffin RE. Spirometric criteria for asthma: adding further evidence to the debate. *J Allergy Clin Immunol*. 2005;116(5):976.
18. Hall GL, Thompson BR, Stanojevic S, et al. The Global Lung Initiative 2012 reference values reflect contemporary Australasian spirometry. *Respirology*. 2012;17(7):1150-1151.
19. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J*. 2012;40(6):1324-1343.
20. Murdzoska J, Devadason SG, Khoo SK, et al. In utero smoke exposure and role of maternal and infant glutathione s-transferase genes on airway responsiveness and lung function in infancy. *Am J Respir Crit Care Med*. 2010;181(1):64-71.
21. Kabesch M, Hoefler C, Carr D, Leupold W, Weiland SK, von Mutius E. Glutathione S transferase deficiency and passive smoking increase childhood asthma. *Thorax*. 2004;59(7):569-573.
22. Bowatte G, Lodge CJ, Lowe AJ, et al. Do variants in GSTs modify the association between traffic air pollution and asthma in adolescence? *Int J Mol Sci*. 2016;17(4):1-13.
23. Hollams EM, de Klerk NH, Holt PG, Sly PD. Persistent effects of maternal smoking during pregnancy on lung function and asthma in adolescents.

Am J Respir Crit Care Med. 2013;189(4):401-407.

24. Fletcher ME, Boshier PR, Wakabayashi K, et al. Influence of glutathione-S-transferase (GST) inhibition on lung epithelial cell injury: role of oxidative stress and metabolism. *Am J Physiol Lung Cell Mol Physiol*. 2015;308(12):L1274-L1285.
25. Li YF, Gilliland FD, Berhane K, et al. Effects of in utero and environmental tobacco smoke exposure on lung function in boys and girls with and without asthma. *Am J Respir Crit Care Med*. 2000;162(6):2097-2104.
26. Bergamaschi E, De Palma G, Mozzoni P, et al. Polymorphism of quinone-metabolizing enzymes and susceptibility to ozone-induced acute effects. *Am J Respir Crit Care Med*. 2001;163(6):1426-1431.
27. David GL, Romieu I, Sienra-Monge JJ, et al. Nicotinamide adenine dinucleotide (phosphate) reduced:quinone oxidoreductase and glutathione S-transferase M1 polymorphisms and childhood asthma. *Am J Respir Crit Care Med*. 2003;168(10):1199-1204.
28. Gilliland FD, Yu-Fen L, Gong JH, Diaz-Sanchez D. Glutathione S-transferases M1 and P1 prevent aggravation of allergic responses by secondhand smoke. *Am J Respir Crit Care Med*. 2006;174(12):1335-1341.
29. Lee YL, Lin YC, Lee YC, Wang JY, Hsiue TR, Guo YL. Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma. *Clin Exp Allergy*. 2004;34(11):1707-1713.
30. Li YF, Gauderman WJ, Conti DV, Lin PC, Avol E, Gilliland FD. Glutathione S-transferase P1, maternal smoking, and asthma in children: a haplotype-based analysis. *Environ Health Perspect*. 2008;116(3):409-415.
31. Schroer KT, Biagini Myers JM, Ryan PH, et al. Associations between multiple environmental exposures and glutathione S-transferase P1 on persistent wheezing in a birth cohort. *J Pediatr*. 2009;154(3):401-408. 408.e401.
32. Schultz EN, Devadason SG, Khoo SK, et al. The role of GSTP1 polymorphisms and tobacco smoke exposure in children with acute asthma. *J Asthma*. 2010;47(9):1049-1056.
33. Munoz B, Magana JJ, Romero-Toledo I, et al. The relationship among IL-13, GSTP1, and CYP1A1 polymorphisms and environmental tobacco smoke in a population of children with asthma in Northern Mexico. *Environ Toxicol Pharmacol*. 2012;33(2):226-232.
34. Sundberg K, Johansson AS, Stenberg G, et al. Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. *Carcinogenesis*. 1998;19(3):433-436.
35. Hu X, Xia H, Srivastava SK, et al. Activity of four allelic forms of glutathione S-transferase hGSTP1-1 for diol epoxides of polycyclic aromatic hydrocarbons. *Biochem Biophys Res Commun*. 1997;238(2):397-402.
36. Francesc CG, Nino K, Bénédicte J, et al. Traffic-Related Air Pollution, Oxidative Stress Genes, and Asthma (ECHRS). *Environ Health Perspect*. 2009;117(12):1919-1924.