

Early life acetaminophen exposure, glutathione S-transferase genes, and development of adolescent asthma in a high-risk birth cohort

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Background: Although the impact of early life acetaminophen on asthma risk is still not clear, potential interactions with glutathione S-transferase (GST) genes due to reduced antioxidant function in particular polymorphisms, and possible impact on lung function, have never been investigated in adolescents.

Objective: We aimed to investigate associations between early life acetaminophen use and adolescent asthma and lung function and to assess potential interactions by GST polymorphisms.

Methods: Acetaminophen use was recorded 18 times up to age 2 years ($n = 575$ [92.7%]). Participants were genotyped for GST polymorphisms (*GSTM1/T1/PI1*) ($n = 429$ [69.2%]). Asthma and lung function were measured at 12 ($n = 365$ [58.9%]) and 18 years ($n = 413$ [66.6%]). Regression models assessed associations and interactions.

Results: Doubling of days of acetaminophen use was associated with reduced prebronchodilator FEV₁/forced vital capacity (β coefficient, -0.10 ; 95% CI, -0.19 to -0.01) and midexpiratory flow (-0.09 ; 95% CI, -0.18 to 0) at 18 years, but this association was not found when restricted for nonrespiratory

reasons, suggesting confounding by indication. However, in children with *GSTM1* null and *GSTT1* present, increasing acetaminophen use for nonrespiratory reasons was associated with reduced FEV₁ and midexpiratory flow at 18 years (interaction between *GSTM1/T1* and acetaminophen $P < .05$). Increased acetaminophen use was associated with asthma at 18 years for children with *GSTP1* Ile/Ile (odds ratio, 1.66; 95% CI, 1.07 to 2.57), but not other *GSTP1* genotypes. **Conclusions:** These novel findings need to be investigated for consistency in other studies but suggest that children carrying risk genotypes may be susceptible to respiratory consequences from acetaminophen use. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: Asthma, oxidative stress, acetaminophen, glutathione transferase, cohort studies, respiratory function tests

Acetaminophen is a commonly used analgesic and antipyretic in young children. Evidence suggests that exposure to acetaminophen in early life may increase the incidence of asthma,^{1,2} but findings are not consistent.^{3,4} This evidence is based on observational studies that have reported that exposure to acetaminophen during pregnancy,^{1,3,5,6} the postnatal period up to 6 months or during the first year of life,^{1,2,7,8} or in childhood^{7,8} is associated with an increased risk of subsequent asthma or wheeze. The hypothesized mechanism for this link is through suppression of glutathione levels. The acetaminophen metabolite *N*-acetyl-*p*-benzo-quinone imine is known to bind with glutathione, depleting the amount available to detoxify reactive oxygen species (ROS).¹ Oxidative stress results from an imbalance between the production of ROS and the capacity of antioxidant defenses. Increased oxidative stress was shown to be associated with airway inflammation that is associated with asthma and poor lung function.⁹ However, the true nature of the association for acetaminophen is not clear due to the potential for confounding by indication, because acetaminophen is commonly used to treat respiratory tract infections, a known risk factor for childhood asthma.

It is plausible that the effects of early life acetaminophen exposure on respiratory outcomes might be modified by the glutathione S-transferase (GST) superfamily of enzymes. The GST family is believed to be important for the prevention of lung damage through the detoxification of ROS generated from inflammation, infection, and/or exposure to airborne toxins.¹⁰ Three major GST enzymes are encoded by the *GSTM1*, *GSTT1* and *GSTP1* genes. Deletion of *GSTM1* and *GSTT1* (recessive model) and *GSTP1* with homozygous Ile genotypes (dominant model) is associated with reduced GST enzyme activity, which may put individuals carrying these genotypes at increased risk

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Abbreviations used

BD:	Bronchodilator
DAG:	Directed acyclic graph
FENO:	Fractional exhaled nitric oxide
FVC:	Forced vital capacity
GST:	Glutathione S-transferase
MACS:	Melbourne Atopy Cohort Study
MEF:	Midexpiratory flow
ROS:	Reactive oxygen species

of respiratory morbidity when exposed to oxidative stress.^{11,12} The possible interactions between GST polymorphisms and acetaminophen use during the first 2 years of life for asthma and lung function outcomes have not been investigated in older adolescents, and airway inflammation was never explored as an outcome.

We hypothesized that this may be due to reduced GST enzyme activity, but acknowledge there was no conclusive evidence found. The objective of this study was to assess the association between acetaminophen use up to the age of 2 years and its interaction with GST genes on asthma, lung function, and airway inflammation at 12 and 18 years in the Melbourne Atopy Cohort Study (MACS). In an earlier analysis using this same cohort, we found no association between acetaminophen use between birth and age 2 years and the risk of asthma at age 7 years after adjustment for respiratory infection, or when acetaminophen use was restricted to nonrespiratory indications.¹³ Our previous work did not examine whether children with genetically reduced oxidative stress management capacity, due to GST polymorphisms, were at increased risk of adverse outcomes when exposed to acetaminophen, and we had not previously addressed lung function outcomes. The present analysis extends our work by investigating potential modification by GST polymorphisms and by following participants up to age 18 years with outcomes of asthma, lung function, and airway inflammation.

METHODS**Study population**

A total of 620 pregnant women were recruited into the MACS between 1990 and 1994 in Melbourne, Australia.¹⁴ Infants had at least 1 first-degree family member with self-reported asthma, eczema, hay fever, or severe food allergy. MACS initially began as a randomized controlled trial of infant formulas,¹⁵ but has since been followed as an observational cohort. Ethics approvals are detailed in this article's Methods section in the Online Repository at www.jacionline.org.

Acetaminophen use

An allergy-trained research nurse conducted telephonic interviews every 4 weeks in the first 15 months, at 18 months, and at age 2 years. Each survey documented illness occurrence and medication use since the last interview. The number of episodes, days of use, and reason for the administration of acetaminophen were recorded at each telephonic interview. The total days of acetaminophen use before age 2 years for each child (regardless of dose or frequency per day) was recorded as the main exposure. In line with our previous analyses,¹³ we classified indications for acetaminophen use into 4 categories: for lower respiratory tract infection/symptom, for upper respiratory tract infection/symptom, for otitis media, and for other reasons (non-respiratory tract infection reasons) (see Table E1 in this article's Online Repository at www.jacionline.org).

Asthma and lung function

Definitions of current asthma at 12 and 18 years are provided in this article's Methods section in the Online Repository. Lung function testing was performed at both 12 and 18 years, following standard techniques described by the American Thoracic Society.^{16,17} Only prebronchodilator (BD) spirometry was measured at 12 years, whereas both pre- and post-BD spirometry were measured at 18 years.

Airway inflammation markers

Fractional exhaled nitric oxide (FENO) was measured at 18 years using Hy-Pair FeNO (Medisoft, Sorinners, Belgium) according to American Thoracic Society/European Respiratory Society guidelines.¹⁸

GST genotyping

Genotyping was performed using Taq-Man allelic discrimination 5' nuclease assays (Applied Biosystems, Foster City, Calif). Deletion of GSTM1 and GSTT1 genotypes was detected by a previously described multiplex PCR technique,¹⁹ and samples of each genotype were sequenced and subsequently used as controls in each run. To ensure that the data were reproducible, 10% of the samples were also genotyped in duplicate. There was a 0% error rate for all single nucleotide polymorphisms analyzed. A customized Illumina GoldenGate Genotyping Assay (www.illumina.com) was used to genotype the GSTP1 (rs1695 A→G: Ile105Val) polymorphism.

Definitions of other variables are detailed in this article's Methods section in the Online Repository.

Statistical analyses

Complete case analysis was performed in this study. Multiple linear and logistic regression were used to assess the relationship between total days of acetaminophen use in the first 2 years of life, GST genotypes, adolescent lung function, and asthma. The distribution of acetaminophen was skewed left (median, 17; mean, 19; interquartile range, 10-27). We applied a log (base 2) transformation with an offset of 1 added for days of acetaminophen exposure. This enabled interpretation of the estimates from the regression models as the effect per doubling of days of acetaminophen use. After transformation, the median log₂ acetaminophen days of exposure was 4 (interquartile range, 3-5).

We selected confounders determined from a directed acyclic graph (DAG) (included in Fig E1 in this article's Online Repository at www.jacionline.org). For asthma and FENO outcomes, the models were adjusted for age, sex, maternal asthma, paternal asthma, and parental education,²⁰ plus frequency of all forms of respiratory infection including lower respiratory tract infection/symptom, upper respiratory tract infection/symptom, and otitis media.²¹ The raw values of lung function were transformed to z scores standardized for age, sex, and height using Global Lung Function Initiative equations,^{22,23} and β coefficients of the z scores were presented when z scores were used as a continuous outcome variable. Lung function outcomes were adjusted for parental education, maternal asthma, paternal asthma, and respiratory infections.

We included all potential confounders defined by a DAG: weight at 4 weeks, gestational age at delivery, breast-feeding, childcare in first 12 months, the presence of older siblings, other infections in the first 2 years, randomized group allocation from the original randomized controlled trial, and parental smoking. However, this model had a substantial proportion of missing data. We then used a second model to include the maximum number of participants by excluding confounders with large degrees of missing information. Both models produced similar findings without changing the estimates more than 10%. We have opted to present the analysis with the smaller set of confounders as the main analysis to maintain the maximum sample size.

To determine whether the indication for use of acetaminophen affected the associations, we investigated the main association between acetaminophen exposure (total use as well as use for nonrespiratory indications only) and respiratory outcomes. Potential GST interactions between these 2 acetaminophen exposures and GST polymorphisms were assessed using likelihood ratio tests. When the likelihood ratio test indicated possible effect modification, GST stratum-specific associations were reported. There is evidence that

GSTM1/T1 null genotypes are associated with lower enzyme activity; thus, individuals were categorized by recessive model as either *GSTM1/T1* null (homozygous for *GSTM1/T1**0 allele) or *GSTM1/T1* present (at least 1 *GSTM1/T1* allele).^{11,12} Because there is also evidence that *GSTP1* with Val alleles may be lower risk genotypes, and no clear evidence of different effects between *GSTP1* with Ile/Ile and Ile/Val alleles on asthma phenotypes,¹⁰ we combined these 2 genotypes in the dominant genetic model. Interactions were considered significant if interaction *P* values were less than .1. All analyses were conducted using Stata, version 14 (2013 StataCorp, College Station, Tex).

RESULTS

Of the 620 infants enrolled, 575 (92.7%) were followed to age 2 years. There were 365 at 12 and 413 at 18 years with spirometry data available (Fig 1). Approximately half the participants were male (Table I). Almost all infants (97%) received at least 1 day of acetaminophen between birth and age 2 years, ranging from 0 to 84 days, with most being exposed for nonrespiratory reasons (93.4%) or for upper respiratory tract infections/symptoms (88.7%) (see Table E2 in this article's Online Repository at www.jacionline.org). Total days of acetaminophen use was not associated with loss to follow-up at 18 years (*P* = .5). The characteristics of the children included in the analyses and those lost to follow-up at 12 and 18 years were not significantly different (see Table E3 in this article's Online Repository at www.jacionline.org). The proportion of participants with current asthma was similar at 12 and 18 years (23.7% [*n* = 85] and 23.5% [*n* = 97], respectively). Between 12 and 18 years, remission of asthma (8.1%) was higher than incidence of asthma (6.4%).

Main associations

Any acetaminophen use and respiratory outcomes.

Asthma status. There was no evidence of an association between doubling total days of acetaminophen use and current asthma at 12 and 18 years (Table II; see Table E4 in this article's Online Repository at www.jacionline.org).

Lung function. However, increased doubling total days of acetaminophen use were associated with reduced pre-BD FEV₁/forced vital capacity (FVC) at 18 years (β coefficient, -0.10 ; 95% CI, -0.19 to -0.01) (Table II). Trends were also seen for modestly reduced pre-BD FEV₁, and midexpiratory flow (MEF) at 18 years. Similarly, we found associations with decreased FEV₁/FVC at 12 years and marginally elevated FVC (see Table E4).

Airway inflammation. There was no evidence of an association between doubling of days of total acetaminophen use and FENO (Table II).

Acetaminophen use for nonrespiratory indications and respiratory outcomes. We found little evidence of an association between acetaminophen use for nonrespiratory reasons and the risk of current asthma, impaired lung function, and FENO. However, all lung function parameter point estimates were negative with increasing doubling days of acetaminophen use and there was a modest association with reduced pre-BD FEV₁/FVC (*P* = .07) (Table II).

Interaction analysis

Any acetaminophen use and respiratory outcomes.

Asthma status. Subjects with *GSTP1* Ile/Ile genotypes were more likely to report current asthma at 18 years if they had

increased doubling of total days of acetaminophen exposure between birth and age 2 years (odds ratio, 1.66; 95% CI, 1.07-2.57; interaction *P* = .02) (Table III). There was no evidence that *GSTM1/T1* polymorphisms modified the association between increased doubling of total days of acetaminophen use and current asthma (Table III). We did not observe significant associations for current asthma at 12 years (see Table E5 in this article's Online Repository at www.jacionline.org).

Lung function. There was little evidence that *GSTM1/T1*/*P1* polymorphisms modified the associations between doubling of total days of acetaminophen use and lung function at 12 and 18 years (see Tables E6-E8 in this article's Online Repository at www.jacionline.org).

Airway inflammation. There was evidence for *GSTP1* interaction on FENO (*P* = .03). In the stratified analysis, the associations between acetaminophen and FENO were increased for participants with *GSTP1* and any Val genotypes compared with homozygous Ile; however, neither of these stratified associations were significant (see Table E9 in this article's Online Repository at www.jacionline.org).

Acetaminophen use for nonrespiratory indications and respiratory outcomes. **Asthma status.** We found little evidence of GST interaction on associations between doubling of days of acetaminophen use for nonrespiratory indications and current asthma (Table III).

Lung function. We have observed a range of consistent results across the analyses for GST interaction on associations between doubling of days of acetaminophen use for nonrespiratory reasons and lung function at both age 12 and 18 years. There was evidence of interactions between doubling of days of acetaminophen use for nonrespiratory indications and *GSTM1* genotypes for FEV₁ and MEF at 18 years (Table IV). Increasing doubling of days of acetaminophen use for nonrespiratory indications in children with *GSTM1* null genotypes was associated with reduced pre- and post-BD lung function, whereas this was not found for children with *GSTM1* present (Fig 2). Similar trends were also found for lung function at 12 years (Table IV). There was also some evidence for a paradoxical finding on pre-BD lung function (FEV₁/FVC, MEF), where increasing doubling of days of acetaminophen use for nonrespiratory reasons was associated with reduced lung function in those with *GSTT1* present, but not in those with *GSTT1* null (Fig 3). Similar trends were also found for lung function at 12 years (Table V). We found no evidence of interaction for *GSTP1* genotypes and doubling of days of acetaminophen use for nonrespiratory indications at 12 or 18 years (see Table E10 in this article's Online Repository at www.jacionline.org).

Airway inflammation. We did not see significant interactions on FENO (see Table E9).

Sensitivity analysis

Our model presented in the main text included confounders determined by a DAG and the completeness of the information from the potential covariates.^{20,21} A sensitivity analysis, performed with the inclusion of all potential confounders, despite missing data, defined by the DAG did not appreciably differ in findings. Results still showed that subjects with *GSTP1* Ile/Ile genotypes were more likely to report asthma at 18 years and that *GSTM1/T1* genotypes modified the associations between

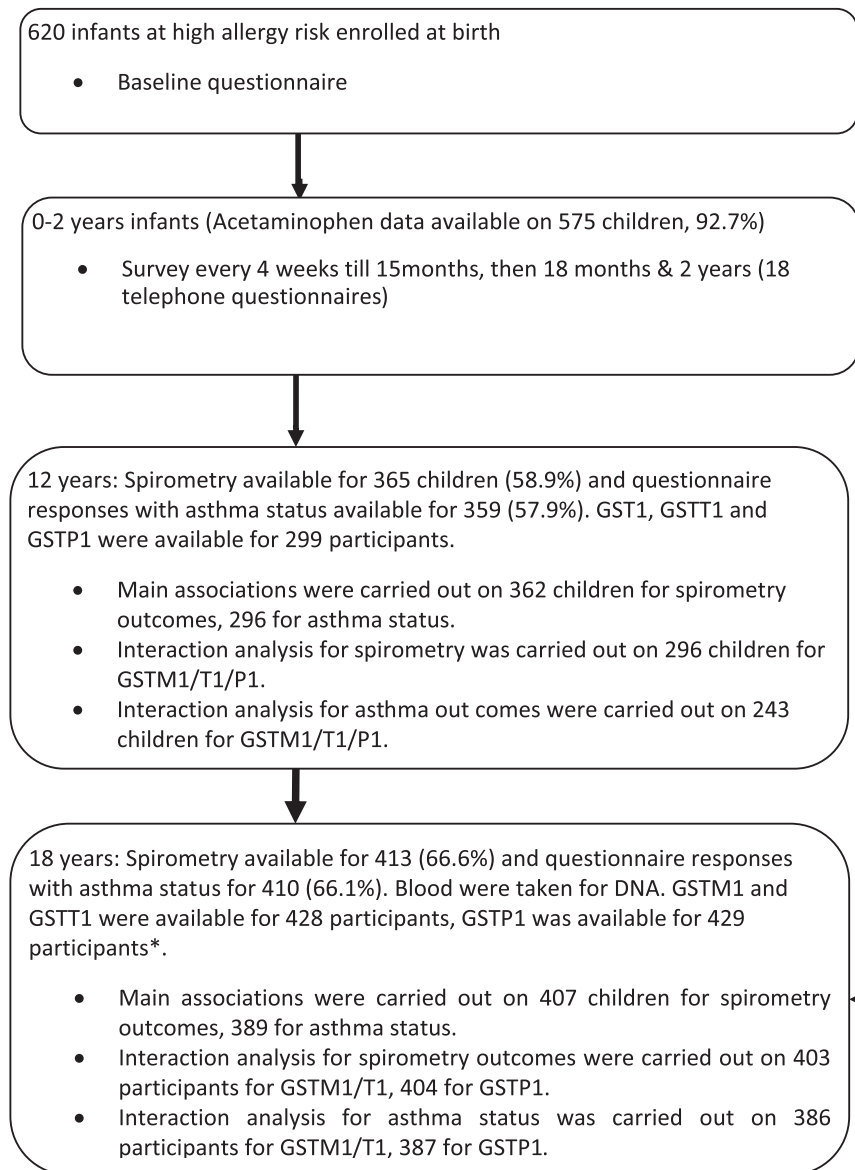


FIG 1. Flowchart of participants in MACS. *There were 21 participants with missing information on asthma.

doubling of days of acetaminophen use for nonrespiratory reasons and lung function at both age 12 and 18 years.

DISCUSSION

This prospective cohort study is the first to assess Gene $\times$ Environment interactions between early postnatal life acetaminophen exposures and GST polymorphisms on associations with asthma and lung function up to early adult life. We found that increasing total doubling of days of acetaminophen use for any indication between birth and age 2 years was associated with reduced FEV₁/FVC ratio at 18 years. When limited to non-respiratory indications, these associations were attenuated. However, we found evidence that GST genotype may modify the association between doubling of days of acetaminophen use and the risk of lung health. Total doubling of days of acetaminophen use was associated with increased risk of asthma for people with

Ile/Ile genotypes, and individuals with *GSTM1* null and *GSTT1* present genotypes were at risk of lower lung function by the age of 18 years when given acetaminophen for nonrespiratory indications, compared with those with *GSTM1* present/*GSTT1* null genotypes.

In a previous analysis of this cohort, we found little evidence of a main association between *GSTM1*/T1 null genotypes for either lung function or current asthma, except that we found some evidence that carriers of *GSTP1* with Ile/Ile genotypes were at an increased risk of reduced post-BD FVC at 18 years compared with those with Ile/Val or Val/Val.²⁴ However, in the current analysis, we found some evidence of the effect of an interaction between acetaminophen use from birth to age 2 years and GST genotype on the risks of asthma and reduced lung function up to age 18 years. These findings are biologically plausible. Glutathione, an important antioxidant, can offer some defense against ROS resulting from environmental stimuli.¹¹ Acetaminophen

TABLE I. Demographic and clinical characteristics of the study population (n = 620)

Characteristic	Initial interview	2-y follow-up	12-y follow-up	18-y follow-up
	N (%)	Mean $\pm$ SD or N (%)	Mean $\pm$ SD or N (%)	Mean $\pm$ SD or N (%)
Total participants	620	575	365	429
Sex				
Male	317 (51.1)	280 (48.7)	189 (51.8)	212 (49.4)
Female	303 (48.9)	295 (51.3)	176 (48.2)	217 (50.6)
Parental completion of a tertiary degree				
Yes	443 (71.4)	417 (72.5)	268 (73.2)	324 (75.5)
No	177 (28.6)	158 (27.5)	98 (26.8)	105 (24.5)
Parental asthma				
Yes	379 (61.2)	348 (60.7)	227 (62.5)	259 (60.7)
No	239 (38.8)	225 (39.3)	136 (37.5)	168 (39.3)
Age (y)			11.50 $\pm$ 1.87	17.98 $\pm$ 1.33
Height (cm)			149.5 $\pm$ 13.0	172.5 $\pm$ 9.3
Current asthma			85 (23.7)	97 (23.5)
Acetaminophen use				
No. of children exposed and % of participants at each follow-up		556 (96.7)	348 (96.9)	402 (97.3)
No. of episodes of exposure, median (IQR)		7 (4-9)	7 (5-9)	7 (4-9)
Total days of exposure, median (IQR)		17 (10-27)	17 (10-27)	17 (9-27)
*Pre-BD lung function, mean for z score				
FEV ₁			-0.3 $\pm$ 1.1	-0.4 $\pm$ 1.1
FVC			-0.7 $\pm$ 1.2	-0.2 $\pm$ 1.0
MEF			-0.8 $\pm$ 1.7	-0.4 $\pm$ 1.1
*Post-BD lung function, mean for z score				
FEV ₁				-0.4 $\pm$ 1.1
FVC				-0.1 $\pm$ 1.0
MEF				0.2 $\pm$ 1.0
FENO				47.75 $\pm$ 46.64
GSTM1			299	428
Present			128 (44)	178 (41.6)
Null			171 (56)	250 (58.4)
GSTT1			299	428
Present			248 (83.6)	353 (82.5)
Null			51 (16.4)	75 (17.5)
GSTP1			299	429
Ile/Ile			132 (44.1)	184 (42.9)
Val/Ile and Val/val			167 (55.9)	245 (57.1)

Variable "parental asthma" has 2 missing data of total participants at each follow-up, respectively; Variable "current asthma" has 18 missing data of total participants at 12 y, 28 missing of 18-y follow-up; GSTM1/T1/P1 have 66 missing data of total participants at 12-y follow-up, GSTM1/T1 have 1 missing data of 18-y follow-up. There was no significant difference between baseline data and data for each follow-up, and data that were missing.

IQR, Interquartile range.

*Lung function was measured using Global Lung Function (GLI) Initiative z scores. These figures represent changes in SD.

reduces the availability of glutathione and therefore reduces a mechanism for managing oxidative stress.²⁵ The lungs, including airways, alveoli, and blood vessels, grow rapidly before age 2 years. Impairment of antioxidant defenses may render individuals susceptible to environmental exposures during this critical window for lung development. Acetaminophen exposure may cause structural lung damage when there is a deficit in the lung's ability to detoxify the oxidant substrates.^{26,27} This may particularly be the case when an exposed individual has *GSTM1/T* null and/or *GSTP1* with Ile/Ile genotypes, because reduced enzyme activity in genetically affected individuals may lead to increased toxicity of acetaminophen metabolites and inability to compete for glutathione.

There are limited studies investigating the interaction effect between infant acetaminophen use and GST genes on respiratory outcomes. One cross-sectional study investigated interactions between acetaminophen use and asthma outcomes only in preschool children (aged 3-7 years) and suggested similar

findings; an adverse effect of acetaminophen use during the first year of life on current asthma was seen only among those with *GSTP1* Ile/Ile and Ile/Val genotypes and not for those with Val/Val genotypes.²⁸ In contrast, a cohort study reported evidence that acetaminophen exposure during pregnancy was associated with increased risk of wheeze at 5 years for those with any Val genotypes, not for those with Ile/Ile genotypes.⁶ Although both studies were suggestive of effect modification, there were some limitations to the evidence, including small numbers of participants with "Val/Val genotypes" and limited adjustment for respiratory tract infection. There was inconsistent evidence for *GSTP1* risk genotypes between studies. Although we cannot explain the inconsistent relationships for *GSTP1*, we suggest that this may arise from failure to account for another common *GSTP1* gene polymorphism, Ala114Val. Such inconsistent results are common in the literature for *GSTP1* variants and respiratory health.²⁰ In contrast, The Avon Longitudinal Study of Parents and Children in the United Kingdom did not identify an interaction effect

TABLE II. Association between doubling of days of acetaminophen use before age 2 y and respiratory outcomes at age 18 y

Respiratory outcome		Total days of acetaminophen use		Days of acetaminophen use for nonrespiratory infection	
		OR	P value	OR	P value
Asthma (n = 389)					
	Current asthma	1.05 (0.83-1.31)	.62	0.96 (0.78-1.17)	.70
		Total days of acetaminophen use		Days of acetaminophen use for nonrespiratory infection	
		Coefficients*	P value	Coefficients	P value
Lung function (n = 407)					
Pre-BD	FEV ₁	−0.07 (−0.16 to 0.01)	.11	−0.06 (−0.14 to 0.03)	.19
	FVC	−0.01 (−0.09 to 0.08)	.82	−0.01 (−0.10 to 0.07)	.80
	FEV ₁ /FVC	−0.10 (−0.19 to −0.01)	.02	−0.08 (−0.17 to 0.01)	.07
	MEF	−0.09 (−0.18 to 0)	.06	−0.07 (−0.16 to 0.02)	.11
Post-BD	FEV ₁	−0.05 (−0.13 to 0.03)	.22	−0.06 (−0.14 to 0.02)	.16
	FVC	−0.02 (−0.10 to 0.06)	.66	−0.03 (−0.12 to 0.05)	.42
	FEV ₁ /FVC	−0.06 (−0.14 to 0.03)	.19	−0.05 (−0.13 to 0.03)	.24
	MEF	−0.04 (−0.13 to 0.04)	.34	−0.06 (−0.14 to 0.03)	.18
FENO (n = 357)		−1.09 (−5.21 to 3.04)	.60	−0.65 (−4.76 to 3.46)	.76

Lung function outcomes were adjusted for parent education, maternal asthma, and paternal asthma plus frequency of respiratory infections, including URTI, LRTI, and OM; asthma additionally also adjusted for age and sex of the participant. FENO outcome was adjusted for age, sex, height at 18 y, parental education, maternal asthma, paternal asthma, and frequency of respiratory infection (URT, LRTI, and OM).

LRTI, Lower respiratory tract infection/symptom; OM, otitis media; OR, odds ratio; URTI, upper respiratory tract infection/symptom. Boldface indicates $P \leq .05$.

*The results for lung function are the β coefficients from linear regression, representing by z score.

TABLE III. Associations between doubling of days of acetaminophen use and asthma outcomes at 18 y stratified by GST genes at 18 y

Asthma outcome		Total days of acetaminophen use						Days of acetaminophen use for nonrespiratory infection					
		N	OR	P value	N	OR	P value	N	OR	P value	N	OR	P value
		<i>GSTP1</i> Ile/val or val/val			<i>GSTP1</i> ile/ile			<i>GSTP1</i> Ile/val or val/val			<i>GSTP1</i> ile/ile		
Current asthma		216	0.84 (0.64-1.09)	.19	171	1.66 (1.07-2.57)	.02	217	0.87 (0.67-1.14)	.31	170	1.18 (0.84-1.65)	.35
		<i>GSTM1</i> present			<i>GSTM1</i> null			<i>GSTM1</i> present			<i>GSTM1</i> null		
Current asthma		158	1.48 (0.92-2.40)	.11	228	0.99 (0.77-1.27)	.54	158	1.25 (0.83-1.86)	.29	228	0.89 (0.69-1.15)	.39
		<i>GSTT1</i> present			<i>GSTT1</i> null			<i>GSTT1</i> present			<i>GSTT1</i> null		
Current asthma		322	1.06 (0.84-1.35)	.60	61	0.84 (0.45-1.55)	.58	323	0.98 (0.79-1.22)	.85	61	0.89 (0.48-1.63)	.70

ORs represent changes per doubling of days of acetaminophen use. *GSTM1/T1* null and *GSTP1* with Ile genotypes are higher risk genotypes. ORs were adjusted for age and sex of the participant, parent education, maternal asthma, paternal asthma, and frequency of respiratory infection (upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media).

OR, Odds ratio.

*P values are the interaction P tested by likelihood ratio test. Boldface indicates $P \leq .05$.

between infant acetaminophen exposure and GST genes on childhood asthma at 7 years and lung function at 8 years. This study collected exposure data on acetaminophen intake for 6 months only, so the total days would be fewer compared with our data, and outcomes were assessed at 7 to 8 years, so this study was potentially investigating earlier development rather than post-diagnosis in our current analysis.⁴ With our more comprehensive methodology, we found evidence that *GSTM1*, T1, and P1 modified the association between doubling of days of acetaminophen use and lung function and current asthma at 12 and 18 years. We found some evidence of an interaction effect between *GSTP1* and acetaminophen use for FENO; however, neither stratum of *GSTP1* polymorphisms showed an association between acetaminophen use and FENO. We postulate that the small sample size may have limited our power to detect significant stratified associations. Our study is the first to assess these interactions in young adults.

Our findings for *GSTT1* were unexpected. We hypothesized that acetaminophen use may be associated with reduced lung

function in *GSTT1* null individuals, but instead found that increasing acetaminophen use for nonrespiratory indications in those with *GSTT1* present was associated with reduced lung function (FEV₁/FVC and MEF) but found no association in those with *GSTT1* null. Interestingly, Shaheen et al⁴ reported similar paradoxical associations in the Avon Longitudinal Study of Parents and Children,⁴ where late gestation exposure to acetaminophen was associated with a higher risk of asthma at 7 years in children whose mothers carried a *GSTT1* present genotype compared with mothers with a null genotype. The reason for these apparently paradoxical associations remains unclear, and these results need to be replicated and the mechanisms explored.

To account for the potential influence of early life respiratory infections, our study and other observational studies^{13,29} investigated the effect of acetaminophen use for nonrespiratory reasons. Like these studies, before taking GST polymorphisms into account we did not find an overall association between acetaminophen use for nonrespiratory reasons and increased risk of adverse

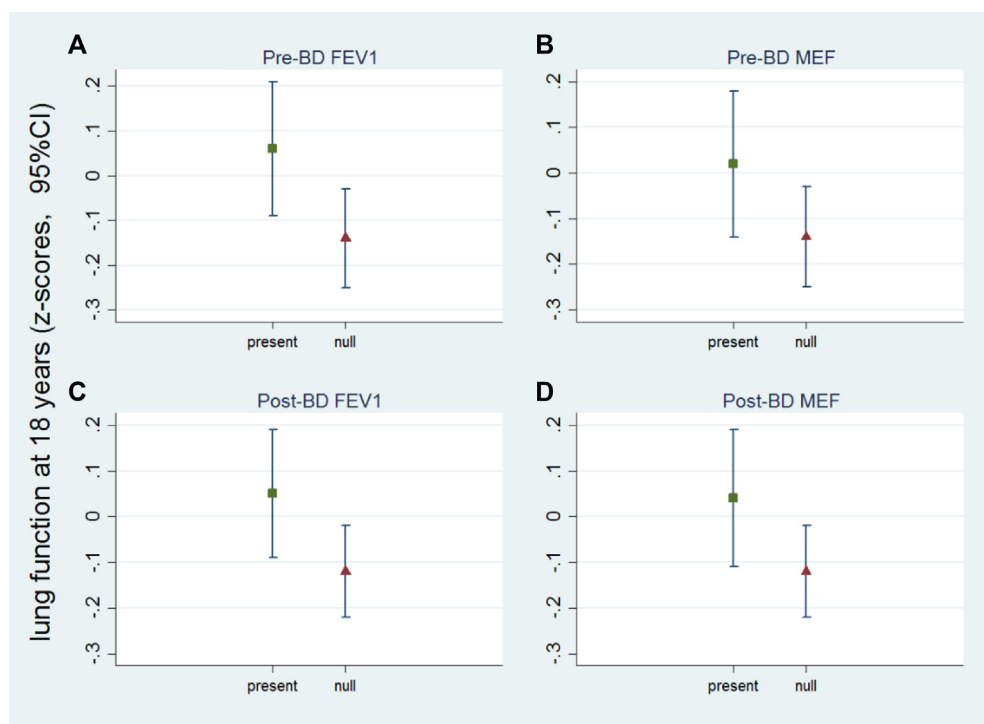
TABLE IV. Associations between days of acetaminophen use for nonrespiratory reasons and lung function (z score) at 12 and 18 y stratified by GSTM1 genotypes

12-y lung function	GSTM1 present		GSTM1 null		Interaction P value
	Coefficients* (n = 126)	P value	Coefficients* (n = 170)	P value	
Pre-BD					
FEV ₁	0.18 (0 to 0.35)	.05	−0.09 (−0.23 to 0.06)	.23	.01
FVC	0.18 (−0.01 to 0.36)	.06	−0.03 (−0.19 to 0.13)	.68	.03
FEV ₁ /FVC	0.01 (−0.20 to 0.23)	.90	−0.14 (−0.31 to 0.04)	.12	.38
MEF	0.09 (−0.19 to 0.37)	.52	−0.14 (−0.36 to 0.08)	.21	.24
18-y lung function					
	GSTM1 present		GSTM1 null		Interaction P value
	Coefficients* (n = 165)	P value	Coefficients* (n = 238)	P value	
Pre-BD					
FEV ₁	0.06 (−0.09 to 0.21)	.43	−0.14 (−0.25 to −0.03)	.02	.01
FVC	0.09 (−0.05 to 0.23)	.22	−0.07 (−0.18 to 0.04)	.22	.05
FEV ₁ /FVC	0.05 (−0.21 to 0.10)	.49	−0.11 (−0.22 to 0)	.05	.42
MEF	0.02 (−0.14 to 0.18)	.80	−0.14 (−0.25 to −0.03)	.01	.04
Post-BD					
FEV ₁	0.05 (−0.09 to 0.19)	.45	−0.12 (−0.22 to −0.02)	.03	.01
FVC	0.06 (−0.08 to 0.19)	.41	−0.08 (−0.19 to 0.03)	.17	.06
FEV ₁ /FVC	0.02 (−0.15 to 0.12)	.82	−0.07 (−0.18 to 0.03)	.19	.38
MEF	0.04 (−0.11 to 0.19)	.57	−0.12 (−0.22 to −0.02)	.02	.02

GSTM1 null is potentially higher risk genotypes.

The results for lung function are the β coefficients from linear regression of z score. Boldface indicates $P \leq .05$.

*Coefficients were adjusted for parent education, maternal asthma, paternal asthma, and frequency of respiratory infections (upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media).

**FIG 2.** The associations between days of acetaminophen use for nonrespiratory reasons and lung function at 18 years stratified by GSTM1 genotypes. The figure only shows the stratified associations for P less than .05. More results can be seen in Table IV.

respiratory outcomes. We also considered that parents who frequently gave acetaminophen to their children for respiratory infection might use more acetaminophen for nonrespiratory reasons; therefore, our associations were also adjusted for frequency

of respiratory infections. However, we observed that doubling of days of acetaminophen use for nonrespiratory indications was associated with increased risk of impaired lung function in young adults with susceptible genotypes.

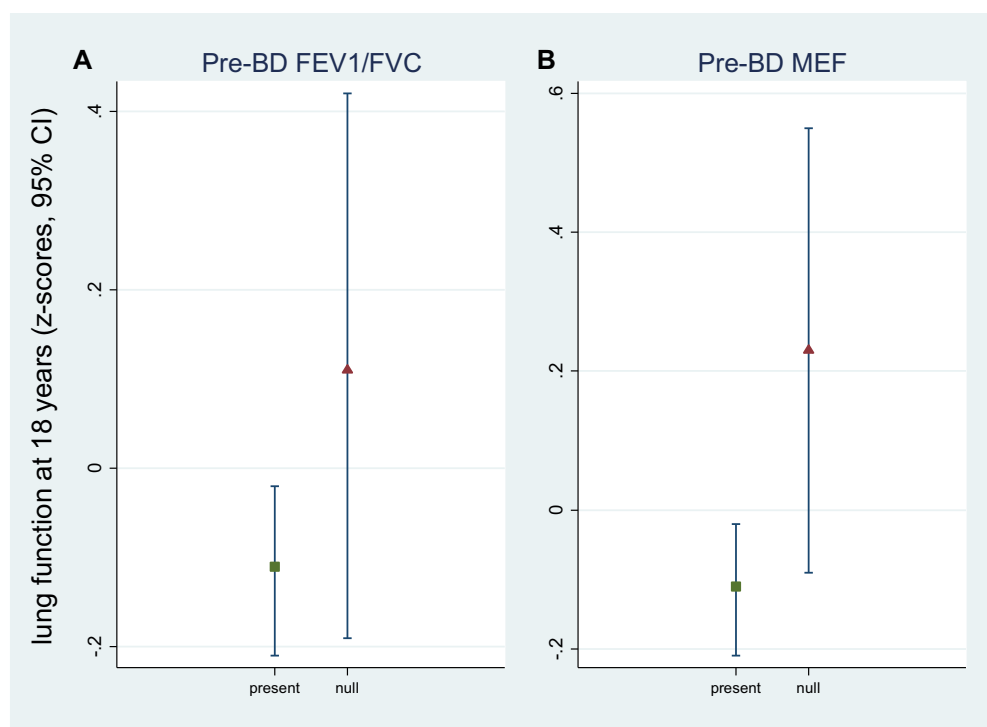


FIG 3. The associations between days of acetaminophen use for nonrespiratory reasons and lung function at 18 years stratified by GSTT1 genotypes. The figure shows the significant associations only. More results can be seen in Table V.

TABLE V. Associations between days of acetaminophen use for nonrespiratory reasons and lung function (z score) at 12 and 18 y stratified by GSTT1 genotypes

12-y lung function	GSTT1 present		GSTT1 null		Interaction P value
	Coefficients* (n = 246)	P value	Coefficients* (n = 50)	P value	
Pre-BD					
FEV ₁	−0.03 (−0.15 to 0.09)	.61	0.26 (−0.11 to 0.63)	.27	.10
FVC	0.03 (−0.09 to 0.16)	.56	0.13 (−0.29 to 0.56)	.80	.80
FEV ₁ /FVC	−0.14 (−0.28 to 0)	.05	0.32 (−0.09 to 0.74)	.13	.01
MEF	−0.11 (−0.29 to 0.07)	.24	0.26 (−0.28 to 0.80)	.26	.12
18-y lung function	GSTT1 present		GSTT1 null		Interaction P value
	Coefficients* (n = 329)	P value	Coefficients* (n = 64)	P value	
Pre-BD					
FEV ₁	−0.08 (−0.17 to 0.01)	.08	0.09 (−0.22 to 0.41)	.55	.46
FVC	−0.02 (−0.11 to 0.07)	.73	0.05 (−0.24 to 0.33)	.75	.87
FEV ₁ /FVC	−0.11 (−0.21 to −0.02)	.02	0.11 (−0.19 to 0.42)	.47	.13
MEF	−0.11 (−0.21 to −0.02)	.02	0.23 (−0.09 to 0.55)	.16	.04
Post-BD					
FEV ₁	−0.04 (−0.13 to 0.05)	.36	−0.17 (−0.42 to 0.09)	.19	.31
FVC	−0.01 (−0.10 to 0.08)	.80	−0.19 (−0.45 to 0.07)	.15	.15
FEV ₁ /FVC	−0.06 (−0.15 to 0.03)	.19	0.04 (−0.22 to 0.30)	.78	.40
MEF	−0.07 (−0.16 to 0.02)	.14	0.06 (−0.20 to 0.32)	.66	.45

GSTT1 null is potentially higher risk genotypes.

The results for lung function are the β coefficients from linear regression of z score. Boldface indicates $P \leq .05$.

*Coefficients were adjusted for parent education, maternal asthma, paternal asthma, and frequency of respiratory infections (upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media).

Our study is a prospective birth cohort, with frequent assessment of acetaminophen use and recorded indications for its use before age 2 years along with objective measurements of respiratory function and inflammation biomarker at 12 and 18

years. All these factors limited the potential for recall bias for acetaminophen exposure in the early years and reduced the potential for measurement error. Puberty influences the risk of asthma and lung function growth.³⁰ We selected 12 and 18 years

as our outcome time points to cover the adolescent period and observe changes. There is an increasing body of evidence highlighting the importance of maximal lung function growth at the end of childhood.³¹⁻³³ Girls usually reach peak lung function by 18 years.³⁰ Suboptimal growth puts individuals at risk of lower lung function trajectories throughout life, and associations with chronic obstructive pulmonary disease and other comorbidities in later life.³⁴ Therefore, evidence of lower relative lung function and lung function growth by 18 years related to modifiable risk factors has important implications.

However, we did not collect data on acetaminophen use by mothers while their children were *in utero*. Prenatal acetaminophen use is believed to be associated with asthma in children,³⁵ and acetaminophen use during pregnancy and early postnatal life may be highly correlated. As such, it is difficult to determine whether postnatal acetaminophen use is only related to lung outcomes through its potential association with pregnancy use by mothers.

As with all long-running cohorts, there is the possibility of bias due to attrition; data were obtained from 66% of the children at the 18-year follow-up. We have previously shown that there were no apparent differences between children lost to follow-up and those who continued to participate for many parameters including sex, family history of allergic diseases, and a range of residential characteristics.¹⁴ Losses to follow-up resulted in smaller numbers of participants at 12 and 18 years and the numbers in each GST stratum reduced the power of our study to detect significance for all associations. Limited sample size may also be responsible for different findings for GST interactions. Because we did not perform correction for multiple comparisons, it is possible that multiple testing may have contributed to the observed associations. However, it should be noted that the associations were based on preestablished hypotheses, and we did not focus on particular estimated effects in isolation, but rather on the trends. All point estimates for associations between children with risk alleles for *GST* genes and respiratory outcomes were consistent in the same direction when exposed to more acetaminophen between birth and age 2 years. We did not use doctor-diagnosed asthma as a definition, because this question was only asked at 18 years. However, there was a high degree of agreement between self-reported asthma and doctor-diagnosed asthma at 18 years in our data, with only 9 participants who reported current asthma, but not doctor-diagnosed asthma. Excluding these 9 participants without doctor-diagnosed asthma did not impact the findings. In addition, because high-risk children with a family history of allergic disease were recruited into the MACS, and the limitation mentioned above, these findings should not be extrapolated to the general population. However, the prevalence of GST polymorphisms did not differ from those in general population.^{11,36} Finally, we did not investigate some alleles with low frequencies, for instance, *GSTP1* Val114Ala, but it is an avenue for further research.

Conclusions

We found some evidence that increased doubling of days of acetaminophen use in the first 2 years of life was associated with reduced lung function in adolescents. There was evidence that this association was confounded by respiratory tract infection, with the models limited to nonrespiratory acetaminophen use finding no robust evidence of reduced lung function. However, we also

found evidence that children with GST genotypes that are associated with reduced antioxidant function may be particularly at risk of reduced lung function in early adulthood when frequently exposed to acetaminophen in early life. We observed that increasing doubling of days of acetaminophen use was associated with increased risk of adverse respiratory health in adolescents with *GSTM1/T1* null and *GSTP1* Ile/Ile polymorphisms for acetaminophen used for nonrespiratory indications and total acetaminophen use, respectively. These findings need to be investigated and corroborated by other studies before translation into clinical practice.

We thank the participants of the MACS. We acknowledge Dr David Hill, Dr Cliff Hosking, and Dr John Thorburn for establishing the MACS and for leading the study up to 12 years. We also acknowledge the contribution from the following investigators and assistants of the 18-year follow-up: Prof Catherine Bennett, A/Prof Lyle Gurrin, Dr Rida Khalafzai, Dr Sharon Goldfeld, Dr Chris Baarton, Dr Vijaya Sundararajan, and A/Prof Mathias Wjst.

Clinical implications: This work contributes to emerging evidence that early life acetaminophen may be a potential contributor to asthma causation and reduced lung function in young adults, particularly in genetically susceptible individuals.

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METHODS

The initial trial arm

The initial study began as a randomized controlled trial aiming to investigate the relationship between infant milk formula and the subsequent risk of allergy. Formula was randomized and introduced as a substitute only if breast-feeding was not possible or was not initiated, or on its cessation, or partial cessation. Participants were randomized into 3 different groups: (1) a standard cow's milk-based formula (NANTM, Nestle, Vevey, Switzerland) as a control group; (2) a partially hydrolyzed formula (NANTM, HA Ibid); or (3) a soy-based formulas (ProSobee; Mead Johnson Nutrition/Bristol Myers, Melbourne, Australia). This randomization was performed only at the time when formula was introduced. We added the trial arm in our analyses as a potential confounder; however, it did not materially change our results, indicating that the randomized intervention did not confound the relationship between acetaminophen use and lung outcomes.

Ethics

The Mercy Maternity Hospital Ethics Committee approved the recruitment and follow-up of the cohort until 12 years. Consent forms were completed by all parents. The 18-year follow-up was approved by the University of Melbourne and the Royal Children's Hospital ethics committees. Informed consent was provided by all participants.

Current asthma

Questionnaire responses by parents were used to define children's asthma status at age 12 years and 18 years. Current asthma at 12 years and 18 years was defined as any episodes of asthma and/or treatment with any asthma medication within the last 12 months. Current asthma was defined by positive answers to the question "Have you ever had asthma?" and either an answer of 1 or more to "How many episodes of asthma have you had in the past 12 months?" and/or a positive answer to "Have you taken any medicines including inhalers or tablets for asthma in the past 12 months?"

Lung function

SpiroCard (PC Spirometer, QRS Diagnostic, Plymouth, Minn) devices were used at 12 years, at which time only pre-BD spirometry was measured.

EasyOne Spirometers (NDD Medical, Inc, Andover, Mass) were used for 18-year follow-up, before and after the inhaled salbutamol via spacer.

Genotyping

Blood or saliva samples were collected from 69.2% of total participants at the 18-year follow-up and DNA extracted ($n = 429$). All assays were performed by a scientist unaware of the clinical status of individual subjects. Genotype assignments were based on 2 consistent experimental results.

Other variables

Baseline data collected at the initial interview (birth) included parental age, parental education (used as a proxy for socioeconomic status), and parental asthma status. Parental education was defined as either mother or father or both having tertiary education at the time of study entry. Parental asthma was defined as either father or mother reporting ever having a history of asthma at baseline. The number of episodes of lower respiratory tract infection/symptom, upper respiratory tract infection/symptom, and otitis media requiring medical attention, regardless of treatment, was classified as 0, 1 to 2, or 3 or more in the first 2 years of life. Parental smoking was defined by either father or mother reporting smoking at baseline; maternal age was made into a binary variable, with the cutoff point at 31 years; the presence of siblings was defined by a positive answer to any older siblings at baseline; breast-feeding was defined by weeks of total breast-feeding; other infection was defined by gastroenteritis, conjunctivitis, or other infections that were not due to respiratory reasons before age 2 years. At the 1-year survey, childcare in the first 12 months of the child's life was defined by a positive answer to the question "Did your child attend childcare in the past 12 months?"

Randomized intervention was classified by 3 groups based on formula introduced at the baseline. The initial study began as a randomized controlled trial and aimed to test the relationship between infant milk formula and the risk of allergy. Formula was introduced as a substitute only if breast-feeding could not be started, or on its cessation, or partial cessation. Participants were assigned into 4 different groups, either: (1) a standard cow's milk-based formula (NANTM, Nestle) as a control group; (2) a partially hydrolyzed formula (NANTM, HA Ibid); or (3) a soy-based formulas (ProSobee, Enfamil, Mead Johnson); (4) exclusive breast-feeding.

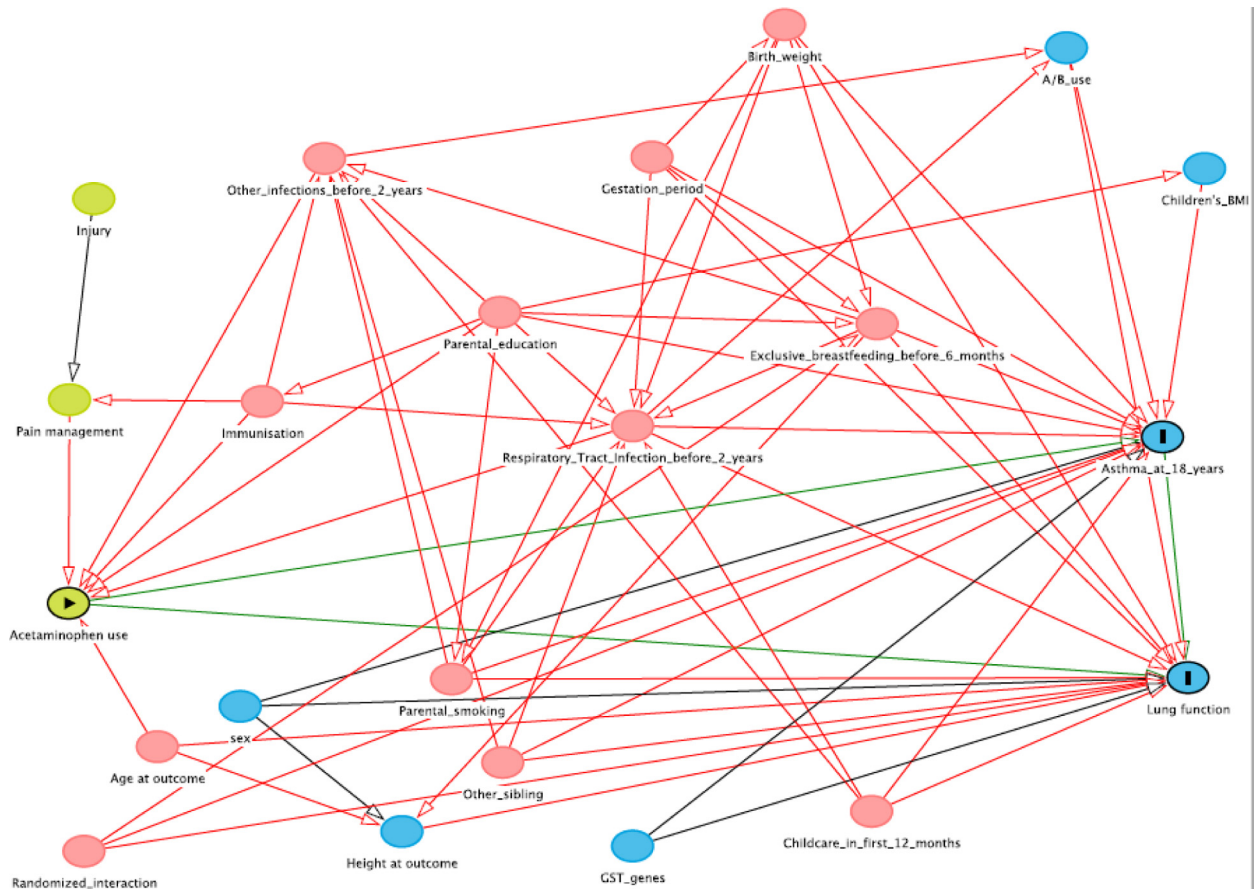


FIG E1. The proposed DAG with full adjustments.

TABLE E1. Classification of indicators for exposure to acetaminophen

Category of acetaminophen indication	Description of indicators
Lower respiratory tract infection	Episodes of acetaminophen exposures due to lower respiratory tract infection, asthma, wheeze of any cause, bronchitis, bronchiolitis, or croup, regardless of any other concomitant reasons for administration
Upper respiratory tract infection/symptoms	Episodes of acetaminophen exposures due to upper respiratory tract infection, tonsillitis, throat infection, regardless of any other concomitant reasons
Otitis media	Otitis media
Others (non–respiratory tract infection reasons)	Episodes of acetaminophen exposures due to eczema or food reaction, regardless of any other concomitant reasons in the category above, gastroenteritis, conjunctivitis, fever without associated evidence of respiratory tract infection, immunizations, or physical injury

TABLE E2. Acetaminophen use up to age 2 y for stated indications (N = 575)

Indication	N (%) children exposed	Episode of exposure, median (IQR)	Cumulative days of exposure, median (IQR)
Any indication	556 (96.7)	7 (4-9)	17 (10-27)
LRI	104 (18.1)	1 (1-1)	4 (2-5)
URI	510 (88.7)	3 (2-5)	9 (5-16)
OM	274 (47.7)	2 (1-2)	5 (3-5)
Nonrespiratory reasons	537 (93.4)	3 (2-5)	7 (4-11)

IQR, Interquartile range; *LRI*, lower respiratory tract infection/symptom; *OM*, otitis media; *URI*, upper respiratory tract infection/symptom.

TABLE E3. The characteristics of the children included in the analyses and those lost to follow-up at 12 and 18 y

Characteristic	12-y follow-up		18-y follow-up	
	Followed-up	Lost to follow-up	Followed-up	Lost to follow-up
N	365	255	429	191
Men, n, (%)	189 (51.8)	128 (50.2)	212 (49.4)	100 (52.4)
Parental completion of a tertiary degree, n (%)	268 (73.2)	173 (67.8)	324 (75.5)	117 (51.3)
Parental asthma, n, (%)	227 (62.5)	152 (59.6)	259 (60.7)	120 (62.8)
Parental smoking at baseline, n (%)	55 (16.1)	43 (18.1)	57 (14.2)	41 (23.0)
Acetaminophen use				
N (%) of children exposed	348 (96.9)	196 (94.2)	402 (97.3)	184 (96.3)
Episode of exposure, median (IQR)	7 (5-9)	6 (4-9)	7 (4-9)	6 (4-9)
Total days of exposure, median (IQR)	17 (10-27)	15 (7-25)	17 (9-27)	14 (7-25)

IQR, Interquartile range.

TABLE E4. Association between total days of acetaminophen use before 2 y and respiratory outcome at 12 y

Asthma	ORs	P value
Current asthma	1.04 (0.80-1.37)	.75

Lung function	Coefficients	P value
FEV ₁	0.02 (−0.08 to 0.13)	.65
FVC	0.10 (−0.01 to 0.21)	.07
FEV ₁ /FVC	−0.16 (−0.28 to −0.04)	.01
MEF	−0.05 (−0.21 to 0.10)	.50

Standard models were adjusted for parent education and parent asthma, with asthma status additionally adjusted for age and sex of the participant. Fully adjusted models were additionally adjusted for frequency of respiratory infections, including upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media. Boldface indicates $P \leq .05$.

OR, Odds ratio.

TABLE E5. Associations between doubling of days of acetaminophen use and current asthma at 12 y stratified by GST genes

Asthma outcome	Total days of acetaminophen use							Days of acetaminophen use for nonrespiratory infections						
	N	OR	P value	N	OR	P value	P value*	N	OR	P value	N	OR	P value	P value*
		<i>GSTP1</i> Ile/val or val/val				<i>GSTP1</i> ile/ile			<i>GSTP1</i> Ile/val or val/val				<i>GSTP1</i> ile/ile	
Current asthma	140	1.07 (0.71 to 1.60)	.75	102	1.47 (0.88 to 2.47)	.14	0.20	140	1.29 (0.86 to 1.93)	.22	103	1.15 (0.71 to 1.88)	.57	.98
		<i>GSTM1</i> present				<i>GSTM1</i> null			<i>GSTM1</i> present				<i>GSTM1</i> null	
Current asthma	140	1.43 (0.83 to 2.49)	.20	102	1.03 (0.71 to 1.50)	.87	0.67	99	1.10 (0.66 to 1.83)	.71	144	1.26 (0.87 to 1.83)	.22	.45
		<i>GSTT1</i> present				<i>GSTT1</i> null			<i>GSTT1</i> present				<i>GSTT1</i> null	
Current asthma	205	1.18 (0.85 to 1.64)	.33	26	0.32 (0.05 to 2.27)	.26	.37	206	1.28 (0.95 to 1.77)	.10	26	0.17 (0.02 to 1.04)	.10	.17

ORs were adjusted for age and sex of the participant, parent education, maternal asthma, paternal asthma, and frequency of respiratory infection (upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media). *GSTM1/T1* null and *GSTP1* with Ile genotypes are higher risk genotypes.

OR, Odds ratio.

**P* values are the interaction *P* tested by likelihood ratio test.

TABLE E6. Associations between total days of acetaminophen use and lung function at 12 and 18 y stratified by GSTM1 genotypes

12-y lung function	GSTM1 present		GSTM1 null		Interaction <i>P</i> value
	Coefficients (n = 126)	<i>P</i> value	Coefficients (n = 170)	<i>P</i> value	
FEV ₁	0.02 (−0.17 to 0.22)	.80	−0.01 (−0.16 to 0.13)	.86	.86
FVC	0.07 (−0.12 to 0.27)	.46	0.08 (−0.08 to 0.24)	.33	.80
FEV ₁ /FVC	−0.09 (−0.31 to 0.14)	.44	−0.21 (−0.38 to −0.04)	.01	.28
MEF	0.16 (−0.14 to 0.46)	.29	−0.15 (−0.36 to 0.07)	.18	.09

18-y lung function	GSTM1 present		GSTM1 null		Interaction <i>P</i> value
	Coefficients (n = 162)	<i>P</i> value	Coefficients (n = 231)	<i>P</i> value	
Pre-BD					
FEV ₁	0.07 (−0.82 to 1.96)	.94	−0.12 (−0.22 to −0.10)	.03	.14
FVC	1.22 (−0.58 to 3.02)	.18	−0.66 (−1.90 to 0.57)	.29	.11
FEV ₁ /FVC	−0.14 (−0.31 to 0.03)	.10	−0.10 (−0.21 to 0)	.06	.93
MEF	−0.07 (−0.24 to 0.09)	.36	−0.11 (−0.21 to 0)	.05	.60
Post-BD					
FEV ₁	0.06 (−0.09 to 0.21)	.41	−0.11 (−0.20 to −0.01)	.03	.03
FVC	0.10 (−0.04 to 0.25)	.15	−0.07 (−0.18 to 0.03)	.18	.05
FEV ₁ /FVC	−0.07 (−0.22 to 0.08)	.37	−0.06 (−0.16 to 0.04)	.27	.78
MEF	0 (−0.16 to 0.17)	.96	−0.07 (−0.17 to 0.02)	.14	.22

ORs were adjusted for age and sex of the participant, parent education, parent asthma, and frequency of respiratory infection (URTI, LRTI and OM). Coefficients were adjusted for parent education, parent asthma, and frequency of respiratory infections (URTI, LRTI, and OM). Boldface indicates $P \leq .05$.

LRTI, Lower respiratory tract infection/symptom; OM, otitis media; OR, odds ratio; URTI, upper respiratory tract infection/symptom.

TABLE E7. Associations between total days of acetaminophen use and lung function at 12 and 18 y stratified by GSTT1 genotypes

	GSTT1 present		GSTT1 null		
12-y lung function	Coefficients (n = 246)	P value	Coefficients (n = 50)	P value	Interaction P value
FEV ₁	−0.02 (−0.14 to 0.10)	.77	0.13 (−0.26 to 0.53)	.50	.04
FVC	0.08 (−0.05 to 0.21)	.21	0.14 (−0.30 to 0.58)	.53	.36
FEV ₁ /FVC	−0.22 (−0.36 to −0.08)	<.01	0.04 (−0.41 to 0.49)	.86	.07
MEF	−0.07 (−0.26 to 0.11)	.45	0.04 (−0.53 to 0.61)	.88	.61

	GSTT1 present		GSTT1 null		
18-y lung function	Coefficients (n = 337)	P value	Coefficients (n = 66)	P value	Interaction P value
Pre-BD					
FEV ₁	−0.07 (−0.16 to 0.02)	.13	−0.04 (−0.34 to 0.26)	.79	.98
FVC	0.01 (−0.08 to 0.10)	.90	−0.02 (−0.29 to 0.25)	.87	.40
FEV ₁ /FVC	−0.13 (−0.22 to −0.03)	.01	−0.03 (−0.30 to 0.29)	.97	.14
MEF	−0.11 (−0.21 to −0.02)	.02	0.07 (−0.24 to 0.38)	.64	.22
Post-BD					
FEV ₁	−0.03 (−0.12 to 0.06)	.54	−0.15 (−0.38 to 0.08)	.20	.34
FVC	0.01 (−0.08 to 0.10)	.81	−0.14 (−0.38 to 0.10)	.24	.15
FEV ₁ /FVC	−0.07 (−0.16 to 0.02)	.14	0 (−0.24 to 0.24)	1.00	.27
MEF	−0.05 (−0.14 to 0.04)	.31	0.03 (−0.21 to 0.27)	.82	.58

ORs were adjusted for age and sex of the participant, parent education, parent asthma, and frequency of respiratory infection (URTI, LRTI, and OM). Coefficients were adjusted for parent education, parent asthma, and frequency of respiratory infections (URTI, LRTI, and OM). Boldface indicates $P \leq .05$.

LRTI, Lower respiratory tract infection/symptom; OM, otitis media; OR, odds ratio; URTI, upper respiratory tract infection/symptom.

TABLE E8. Associations between total days of acetaminophen use and lung function (z score) at 12 and 18 y stratified by GSTP1 genotypes

Genotypes	GSTP1 Ile/val or val/val		GSTP1 ile/ile		Interaction <i>P</i> value
	Coefficients (n = 165)	<i>P</i> value	Coefficients (n = 131)	<i>P</i> value	
12-y lung function					
FEV ₁	0.01 (−0.15 to 0.16)	.93	0.02 (−0.16 to 0.20)	.84	.90
FVC	0.11 (−0.05 to 0.26)	.17	0.08 (−0.13 to 0.29)	.51	.59
FEV ₁ /FVC	−0.19 (−0.36 to −0.02)	.03	−0.18 (−0.40 to 0.05)	.12	.66
MEF	−0.04 (−0.24 to 0.17)	.71	−0.11 (−0.42 to 0.20)	.49	.98
18-y lung function					
Pre-BD					
FEV ₁	−0.08 (−0.20 to 0.04)	.21	−0.08 (−0.21 to 0.05)	.23	.91
FVC	−0.02 (−0.13 to 0.10)	.79	−0.03 (−0.17 to 0.11)	.79	.89
FEV ₁ /FVC	−0.09 (−0.21 to 0.02)	.11	−0.12 (−0.27 to 0.03)	.13	.50
MEF	−0.09 (−0.21 to 0.03)	.13	−0.09 (−0.24 to 0.06)	.25	.97
Post-BD					
FEV ₁	−0.07 (−0.18 to 0.04)	.23	−0.03 (−0.17 to 0.11)	.67	.88
FVC	−0.03 (−0.14 to 0.08)	.54	−0.01 (−0.15 to 0.12)	.84	.71
FEV ₁ /FVC	−0.05 (−0.15 to 0.06)	.40	−0.04 (−0.18 to 0.10)	.53	.59
MEF	−0.06 (−0.16 to 0.05)	.31	0 (−0.14 to 0.15)	.98	.77

Coefficients were adjusted for parent education, parent asthma, and frequency of respiratory infections (upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media). Boldface indicates $P \leq .05$.

TABLE E9. Associations between acetaminophen use and FENO stratified by GST genes at 18 y

Total days of acetaminophen use								Days of acetaminophen use for nonrespiratory infections						
	N	Coefficients	P value	N	Coefficients	P value	P value*	N	Coefficients	P value	N	Coefficients	P value	P value*
		<i>GSTP1 Ile/val or val/val</i>			<i>GSTP1 ile/ile</i>				<i>GSTP1 Ile/val or val/val</i>			<i>GSTP1 ile/ile</i>		
FENO	190	2.69 (−2.26 to 7.64)	.29	165	−5.49 (−13.04 to 2.06)	.15	.03	190	1.41 (−3.55 to 6.37)	.58	165	−2.50 (−9.91 to 4.91)	.51	.21
		<i>GSTM1 present</i>			<i>GSTM1 null</i>				<i>GSTM1 present</i>			<i>GSTM1 null</i>		
FENO	147	−2.04 (−11.01 to 6.93)	.65	207	−1.52 (−6.01 to 2.98)	.51	.68	147	0.43 (−7.53 to 8.40)	.91	207	−1.95 (−6.74 to 2.83)	.42	.77
		<i>GSTT1 present</i>			<i>GSTT1 null</i>				<i>GSTT1 present</i>			<i>GSTT1 null</i>		
FENO	294	−0.65 (−5.23 to 3.93)	.78	60	−1.70 (−12.44 to 0.02)	.75	.73	294	0.28 (−4.23 to 4.78)	.90	60	−6.76 (−17.80 to 4.28)	.22	.12

Coefficients for FENO were adjusted for age, sex, height at 18 y, parental education, maternal asthma, paternal asthma, and frequency of respiratory infection (upper respiratory tract infection/symptom, lower respiratory tract infection/symptom, and otitis media).

*Interaction *P* values.

TABLE E10. Associations between days of acetaminophen use for nonrespiratory infection reasons and respiratory outcomes at 12 and 18 y stratified by GSTP1 genotypes

	GSTP1 Ile/val or val/val		GSTP1 ile/ile		
12-y lung function	Coefficients (n = 165)	P value	Coefficients (n = 131)	P value	Interaction P value
FEV ₁	0.04 (−0.11 to 0.18)	.64	−0.02 (−0.21 to 0.17)	.83	.57
FVC	0.08 (−0.07 to 0.23)	.28	0.01 (−0.21 to 0.23)	.94	.46
FEV ₁ /FVC	−0.09 (−0.25 to 0.08)	.31	−0.09 (−0.33 to 0.14)	.44	.97
MEF	0 (−0.19 to 0.20)	.98	−0.25 (−0.57 to 0.07)	.13	.37

	GSTP1 Ile/val or val/val		GSTP1 ile/ile		
18-y lung function	Coefficients (n = 227)	P value	Coefficients (n = 177)	P value	Interaction P value
Pre-BD					
FEV ₁	−0.05 (−0.18 to 0.07)	.38	−0.08 (−0.21 to 0.05)	.24	.81
FVC	0 (−0.10 to 0.12)	.87	−0.07 (−0.21 to 0.07)	.32	.43
FEV ₁ /FVC	−0.10 (−0.22 to 0.01)	.07	−0.05 (−0.20 to 0.10)	.54	.60
MEF	−0.09 (−0.21 to 0.02)	.12	−0.05 (−0.20 to 0.10)	.53	.61
Post-BD					
FEV ₁	−0.02 (−0.12 to 0.09)	.67	−0.11 (−0.25 to 0.03)	.11	.33
FVC	0.01 (−0.10 to 0.12)	.90	−0.11 (−0.24 to 0.03)	.12	.24
FEV ₁ /FVC	−0.04 (−0.15 to 0.06)	.43	−0.03 (−0.17 to 0.11)	.66	.92
MEF	−0.05 (−0.16 to 0.06)	.37	−0.05 (−0.19 to 0.10)	.52	.84

ORs were adjusted for age and sex of the participant, parent education, parent asthma, and frequency of respiratory infection (URTI, LRTI and OM).

LRTI, Lower respiratory tract infection/symptom; OM, otitis media; OR, odds ratio; URTI, lower respiratory tract infection/symptom.

Coefficients were adjusted for parent education, parent asthma, and frequency of respiratory infections (URTI, LRTI, and OM).