

Prenatal and infant acetaminophen exposure, antioxidant gene polymorphisms, and childhood asthma

Seif O. Shaheen, PhD,^a Roger B. Newson, DPhil,^a Susan M. Ring, PhD,^b Matthew J. Rose-Zerilli, BSc,^d John W. Holloway, PhD,^d and A. John Henderson, MD^c London, Bristol, and Southampton, United Kingdom

Background: Prenatal and infant acetaminophen exposure has been associated with an increased risk of childhood asthma phenotypes. Demonstration of biologically plausible interactions between these exposures and maternal and child antioxidant gene polymorphisms would strengthen causal inference.

Objective: To explore potential interactions between prenatal and infant acetaminophen exposure and antioxidant genotypes on childhood asthma.

Methods: In the Avon Longitudinal Study of Parents and Children, we typed a functional nuclear erythroid 2 p45-related factor 2 (*Nrf2*) polymorphism and glutathione S-transferase (*GST*) *M1*, *T1*, and *P1* polymorphisms. Effects of prenatal and infant acetaminophen exposure on asthma phenotypes at 7 years were stratified by genotype in >4000 mothers and >5000 children.

Results: Risk of asthma and wheezing associated with early gestation acetaminophen exposure was increased when maternal copies of the minor T allele of *Nrf2* were present (*P* interactions, .02 and .04, respectively). Risk of asthma associated with late gestation exposure was higher when maternal *GSTT1* genotype was present rather than absent (*P* interaction, .006), and risk of wheezing was increased when maternal *GSTM1* was present (*P* interaction, .04). Although acetaminophen use in infancy was associated with an increased risk of atopy, child antioxidant genotype did not modify

associations between infant acetaminophen use and asthma phenotypes. However, the increased risk of asthma and wheezing associated with late gestation acetaminophen exposure in the presence of maternal *GSTM1* was further enhanced when *GSTM1* was also present in the child.

Conclusion: Maternal antioxidant gene polymorphisms may modify the relation between prenatal acetaminophen exposure and childhood asthma, strengthening evidence for a causal association. In contrast, relations between infant acetaminophen use and asthma and atopy were not modified by child genotype and may be confounded by pre-existing wheeze or allergy.

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Key words: Asthma, acetaminophen, paracetamol, glutathione-S-transferase, *Nrf2*, prenatal exposure, delayed effects, ALSPAC, pregnancy, birth cohort, genotype, gene-environment interaction

Following our original observations in adults and children,^{1,2} a large body of epidemiologic evidence has accumulated linking acetaminophen (paracetamol) exposure throughout the life course to asthma.³ However, the majority of studies that have reported associations between infant or childhood acetaminophen use and childhood asthma have been cross-sectional, limiting causal interpretation. It is possible that the associations may be explained by confounding by indication, whereby children with wheezing associated with febrile viral respiratory infections will be given acetaminophen as an antipyretic. Furthermore, the largest of these studies made inferences about infant exposure on the basis of recall by mothers 5 years later.⁴ Large prospective birth cohort studies are needed to draw more reliable conclusions about the relationship of infant acetaminophen use to later childhood asthma. Recently no association was found between use in infancy or early childhood and wheezing at 5 years in a small US birth cohort.⁵ Longitudinal studies have found more compelling evidence to implicate prenatal exposure in the inception of childhood asthma. In a large population-based birth cohort study, the Avon Longitudinal Study of Parents and Children (ALSPAC), we reported that maternal use of acetaminophen in pregnancy was associated with an increased risk of early childhood wheezing⁶ and of asthma and wheezing at 6 years and elevated total IgE at 7 years, but not atopy.⁷ An association between prenatal acetaminophen exposure and increased risk of early wheezing and later asthma has since been confirmed in the large Danish National Birth Cohort,⁸ and 2 small US studies have recently confirmed an association with wheezing in infancy⁹ and in childhood,⁵ the latter also reporting an association with atopy. We proposed that, if causal, the associations with prenatal exposure might be explained by increased oxidative stress and depletion

From ^athe Respiratory Epidemiology and Public Health Group, National Heart and Lung Institute, Imperial College London; the Departments of ^bSocial Medicine and ^cCommunity-based Medicine, University of Bristol; and ^dthe Respiratory Genetics Group, Division of Infection, Inflammation and Immunity and Division of Human Genetics, University of Southampton.

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Reprint requests: Seif O. Shaheen, PhD, MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, Centre for Health Sciences, Barts and The London School of Medicine and Dentistry, 2 Newark Street, Whitechapel, London, E1 2AT, United Kingdom. E-mail: s.shaheen@qmul.ac.uk.

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Abbreviations used

ALSPAC: Avon Longitudinal Study of Parents and Children
 BMI: Body mass index
 BR: Bronchial responsiveness
 FEF₂₅₋₇₅: Forced expiratory flow at 25% to 75% of forced vital capacity
 FVC: Forced vital capacity
 GST: Glutathione-S-transferase
 NAPQI: *N*-acetyl-*p*-benzoquinoneimine
 Nrf2: Nuclear erythroid 2 p45-related factor 2
 OR: Odds ratio
 SNP: Single nucleotide polymorphism

of glutathione.^{6,7} If true, one would expect the effect to be modified by maternal antioxidant gene polymorphisms, which might influence acetaminophen toxicity. The transcription factor nuclear erythroid 2 p45-related factor 2 (*Nrf2*, also known as *NFE2L2*) is a master regulator of antioxidant and detoxifying genes such as the glutathione-S-transferases (*GSTs*) and protects the lung against oxidative stress by binding to antioxidant response elements of these genes and upregulating their expression.¹⁰ Furthermore, *Nrf2* knockout mice are particularly sensitive to acetaminophen hepatotoxicity.¹¹⁻¹³ The *GSTs* also play a role in detoxification of the oxidative metabolite of acetaminophen, *N*-acetyl-*p*-benzoquinoneimine (NAPQI), through conjugation with glutathione, and *GSTP1* has been shown to influence acetaminophen hepatotoxicity in knockout mice.¹⁴

Previous cross-sectional studies of infant acetaminophen exposure have not had information on prenatal exposure to determine whether effects of the latter might confound effects of infant use. In the longitudinal ALSPAC cohort, we have therefore examined whether effects of prenatal and infant acetaminophen exposure on childhood asthma phenotypes are independent of each other. To strengthen causal inference, we have also explored whether effects of prenatal and infant acetaminophen exposure are modified by *Nrf2* and *GST* polymorphisms in the mother and child.

METHODS**Subjects**

The ALSPAC is a population-based birth cohort that recruited 14,541 pregnant women resident in Avon, United Kingdom, with expected dates of delivery April 1, 1991, to December 31, 1992. There were 14,062 live-born children, and 13,988 of these children were alive at age 1 year and subsequently followed up. The cohort has been followed since birth with annual questionnaires and since age 7 years with objective measures in annual research clinics. The study protocol has been described previously,¹⁵ and further information can be found at <http://www.alspac.bris.ac.uk>. Ethics approval for all aspects of data collection was obtained from the ALSPAC Law and Ethics Committee (IRB 00003312).

Exposures

Mothers were asked at 18 to 20 weeks how often they had taken acetaminophen ("not at all," "sometimes," "most days," "every day") during their pregnancy. At 32 weeks they were asked the same question about use in the previous 3 months. Because very few mothers reported daily use, we combined this category with use on "most days." Thus we defined use of acetaminophen in early (<18-20 weeks) and late (20-32 weeks) pregnancy. At 6 months after birth, mothers were asked how often they had given their infant acetaminophen since birth ("never," "once," "more than once").

Outcomes

When the children were 7.5 years old, mothers were asked "Has your child had any of the following in the past 12 months: wheezing; asthma; eczema; hay fever?" Children were defined as having current doctor-diagnosed asthma at 7.5 years (primary outcome of interest) if mothers responded positively to the question "Has a doctor *ever* actually *said* that your study child has asthma?" and positively to 1 or both of the questions on wheezing and asthma in the past 12 months.

Atopy at 7 years was defined as a positive reaction (maximum diameter of any detectable weal) to *Dermatophagoides pteronyssinus*, cat, or grass (after subtracting positive saline reactions from histamine and allergen wheals and excluding children unreactive to 1% histamine). This was used to define atopic and nonatopic asthma. Serum total IgE (kU/L) was measured by fluorimunoassay using the Pharmacia UNICAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Lung function was measured by spirometry (Vitalograph 2120; Vitalograph, Maids Moreton, England) at age 8.5 years after withholding short-acting bronchodilators for at least 6 hours and long-acting bronchodilators and theophyllines for at least 24 hours. The best of 3 reproducible flow-volume curves was used to measure FEV₁, forced vital capacity (FVC), and maximal mid-expiratory flow (forced expiratory flow at 25% to 75% of FVC [FEF₂₅₋₇₅]). Lung function measurements were transformed to age-adjusted, height-adjusted, and sex-adjusted SD units.¹⁶ Bronchial responsiveness (BR) to methacholine was measured by using the method of Yan.¹⁷ BR was expressed as the dose-response slope (% FEV decline/ μ mole methacholine; see this article's Methods section in the Online Repository at www.jacionline.org for further details).

Confounders

For each acetaminophen exposure (early and late gestation and infancy), we defined propensity scores¹⁸ by using ordinal logistic regression models with each exposure as the predicted variable and a list of confounders as predictive factors. The propensity score is a summary measure of the "acetaminophen-proneness" of mothers and infants, based on a list of confounding variables, and is used to model out the collective and cumulative confounding effect of those confounders as completely as possible, without attempting to measure individual confounder effects on the outcome. The confounders were maternal factors during pregnancy (smoking, infections, anxiety score, antibiotic use, and alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, body mass index (BMI), ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7 years). For analyses of prenatal acetaminophen exposure, we controlled for acetaminophen use in infancy, and for analyses of infant acetaminophen exposure, we controlled for maternal acetaminophen use in pregnancy and also for use of antibiotics in the first 6 months after birth. Details of confounder categories are given in this article's Table E1 in the Online Repository at www.jacionline.org. For each propensity score, the cohort was split into propensity percentile groups of roughly equal size (32 groups for prenatal exposure and 16 groups for infant exposure). An advantage of the propensity score approach is that it reduces the number of model parameters, thus preventing potential nonconvergence of the regression models.

Genotyping

The majority of maternal DNA samples were extracted from whole blood and white cells taken during pregnancy, and a minority were buccal DNA extracted from mouthwash samples. The majority of the children's DNA samples were extracted from cord blood or venous blood collected at age 7 years, with a small number extracted from venous blood collected at 43 to 61 months. Two single nucleotide polymorphisms (SNPs) were typed by KBiosciences Ltd (Hoddesdon, Herts, United Kingdom; <http://www.kbioscience.co.uk>) using a competitive allele-specific PCR system (KASPar): a SNP in the promoter region of *Nrf2* (-684/-651 G/A, rs6706649) and a SNP in *GSTP1* (G313A,

Ile105Val, rs1695). The former has been previously typed in a Japanese population¹⁹ and recently in ethnically diverse individuals.²⁰ In our data, the *Nrf2* SNP was typed by using the reverse strand (C/T). The *GSTT1* and *GSTM1* gene deletion genotyping was performed in Southampton (M. J. R.-Z.) by using a real-time PCR method described previously.²¹ This enabled measurement of copy number variation with identification of hemizygotes in addition to classifying as gene present or absent. Genotyping failure rates and error rates based on duplicate samples for mothers and children are shown in this article's Table E2 in the Online Repository at www.jacionline.org.

Statistical analyses

Acetaminophen exposures were analyzed as linear per category effects by using regression (logistic for binary outcomes, linear on the logs for total IgE and BR slope, and untransformed linear for lung function outcomes). Confidence limits were calculated by using Huber variances.²² The confounder-adjusted effects of acetaminophen were stratified by maternal and child *Nrf2* and *GST* genotypes. To test for gene-acetaminophen interaction, we used a Wald χ^2 or F-test for heterogeneity between the acetaminophen effects in the genotype strata. Because the number of individuals with the homozygous minor allele for *Nrf2* was very small (leading to infinite odds ratios for some analyses), we combined these individuals with heterozygotes.

RESULTS

Of 13,118 mothers with data on acetaminophen use in early pregnancy, 45% did not take the drug at all, 53% took it sometimes, and 2% took it most days/daily. Corresponding figures for the 12,127 mothers with data for use in late pregnancy were 56%, 43%, and 1%. Table I shows the effects of acetaminophen use in early and late pregnancy on respiratory and atopic outcomes in the child after controlling for confounders in individuals with complete outcome data. Early and late gestation exposures were associated with an increased risk of asthma and wheezing, although the effect of late gestation exposure on wheezing was stronger, and only late gestation exposure was associated with elevated IgE. Although mutual adjustment of the effects of early and late gestation exposure on asthma and wheezing led to attenuation of effect estimates, evidence for independent effects of each exposure remained, and we therefore kept analysis of the 2 exposures separate. If we omitted variables from the model that could theoretically be on the causal pathway (gestational age,²³ birth anthropometry, and child's BMI at age 7 years), and therefore should not be controlled for,²⁴ the effects of prenatal acetaminophen on asthma and wheezing were even stronger (data not shown). The associations with asthma were limited to the nonatopic phenotype; no association was seen with atopic asthma (data not shown). There were no associations with lung function or BR.

Table II shows the relation between acetaminophen use in early and late pregnancy and childhood asthma, stratified by maternal *Nrf2* and *GST* genotypes. Risk of asthma increased when mothers had 1 or 2 copies of the minor T allele of the *Nrf2* promoter SNP. This was particularly evident for early gestation exposure (*P* interaction, .02). There was also evidence of effect modification by *GSTT1*, especially for late gestation exposure, with increased risk of asthma occurring in the presence of 1 or 2 copies, but not when *GSTT1* was null (*P* interaction for null genotype, .006). Similarly, risk was increased in the presence of 2 copies of *GSTM1*. There was no convincing evidence for modification by maternal *GSTP1*.

Table III shows associations of prenatal acetaminophen exposure with childhood wheezing stratified by maternal genotype. As for asthma, risk of wheezing was increased when the minor

T allele of the *Nrf2* promoter SNP was present (*P* interaction, .04). There was also evidence of modification of the effect of late gestation exposure on wheezing by *GSTT1* and *GSTM1* null genotypes (*P* interaction, .08 and .04, respectively). There was no evidence of interaction between maternal *GSTP1* genotype and prenatal acetaminophen on wheezing.

This article's Tables E3 and E4 in the Online Repository at www.jacionline.org show the relations of early and late gestation acetaminophen exposure to asthma and wheezing, stratified by child genotype. There was no evidence for interaction with *Nrf2* genotype. In keeping with the findings for maternal *GSTM1*, the risk of asthma and wheezing was highest when 2 copies of *GSTM1* were present in the child, although evidence for interaction was weak. Risk of asthma and wheezing associated with late gestation acetaminophen exposure was enhanced further when *GSTM1* was present in both mother and child (odds ratio [OR] for asthma [*n* = 1103], 1.73; 95% CI, 1.17-2.55; *P* = .006; OR for wheeze [*n* = 1119], 1.67; 95% CI, 1.12-2.49; *P* = .01). Child *GSTP1* genotype did not modify the effects of prenatal acetaminophen exposure on asthma or wheezing. When we examined other phenotypes, we found that maternal (but not child) *GSTT1* null genotype modified the effect of early and late gestation exposure on atopy (*P* interaction, .02 and .003, respectively), with increased risk when *GSTT1* was present (OR for late gestation exposure, 1.19; 95% CI, 0.99-1.42; *n* = 2969), and reduced risk when the genotype was null (OR, 0.64; 95% CI, 0.41-1.01; *n* = 577). There was also evidence that maternal *Nrf2* genotype modified the effect of late gestation exposure on risk of eczema (*P* interaction, .03). There were no interactions between maternal or child genotype and prenatal acetaminophen exposure on total IgE.

Of 11,438 infants with data on infant acetaminophen use, 14% were not given the drug in the first 6 months after birth, 20% were given it once, and 66% were given it on 2 or more occasions. Table IV shows the unadjusted and adjusted effects of acetaminophen use in the first 6 months of infancy on childhood respiratory and atopic outcomes. When we controlled just for prenatal acetaminophen exposure, the association between infant use and asthma was attenuated a little (OR reduced from 1.19 to 1.16), whereas the relative change in effects of prenatal exposure was less on controlling for infant exposure (OR for early gestation exposure reduced from 1.36 to 1.33, and OR for late gestation exposure from 1.45 to 1.42). After controlling for all confounders, infant use was associated with an increased risk of childhood asthma, wheezing, and atopy.

When we stratified the adjusted infant acetaminophen analyses according to whether the child wheezed or not in the first 6 months, we found that the risks of wheezing, eczema, and especially asthma, but not atopy, were greater in children who wheezed in infancy (Table V). Table VI shows the associations of infant acetaminophen exposure with asthma, stratified by child genotype. There was no evidence for interaction with any of the *GST* or *Nrf2* genotypes. Similarly, there was no evidence of effect modification of effects of infant acetaminophen use on wheezing and atopy by child genotype (see this article's Tables E5 and E6 in the Online Repository at www.jacionline.org).

DISCUSSION

Effects of prenatal acetaminophen exposure

In this study we have extended our previous observations⁷ and found some evidence for effect modification of the effect of

TABLE I. Adjusted effects of maternal acetaminophen use in early and late pregnancy on asthma and related phenotypes

Outcome	Early pregnancy				Late pregnancy			
	N	Adj. estimate*	(95% CI)	P value	N	Adj. estimate*	(95% CI)	P value
OR								
Asthma	7929	1.25	(1.09, 1.44)	.001	7673	1.29	(1.12, 1.49)	<.001
Wheezing	8019	1.18	(1.02, 1.36)	.03	7758	1.26	(1.09, 1.47)	.002
Eczema	8005	1.00	(0.89, 1.13)	.97	7743	1.06	(0.93, 1.20)	.4
Atopy	6373	0.97	(0.85, 1.09)	.57	6113	1.00	(0.88, 1.14)	.98
Geometric mean ratio								
BHR slope	4260	1.03	(0.93, 1.13)	.6	4122	1.04	(0.94, 1.15)	.49
Total IgE	5021	1.02	(0.93, 1.11)	.75	4848	1.14	(1.04, 1.26)	.007
Arithmetic mean difference (SD units)								
Adjusted FEV ₁	6436	−0.01	(−0.06, 0.04)	.82	6218	0.00	(−0.05, 0.05)	.87
Adjusted FVC	6536	−0.01	(−0.06, 0.04)	.64	6314	−0.02	(−0.07, 0.03)	.55
Adjusted FEF ₂₅₋₇₅	6536	0.01	(−0.04, 0.06)	.67	6314	0.03	(−0.02, 0.08)	.24

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, antibiotic use, and alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7 years, infant acetaminophen use).

prenatal acetaminophen exposure on risk of doctor-diagnosed asthma and wheezing by maternal *Nrf2* and *GST* genotypes.

Nrf2 was of *a priori* interest for 2 reasons. First, knockout of this gene in mice increases susceptibility to acetaminophen hepatotoxicity.¹¹⁻¹³ Second, it has a central role in induction of antioxidant gene transcription, defending the lung against oxidative stress¹⁰ and protecting against pulmonary disease.²⁵ Disruption of *Nrf2* leads to severe allergen-driven airway inflammation, hyperresponsiveness, and increased T_H2 cytokine levels in a mouse model of asthma²⁶ and, in human beings, polymorphisms in *Nrf2* have recently been linked to adult lung function²⁷ and to the risk of acute lung injury.²⁰ We propose that the risk of asthma and wheezing associated with prenatal acetaminophen exposure is enhanced in the presence of the minor allele of the *Nrf2* promoter SNP because antioxidant defenses are compromised; the minor allele has been associated with reduced expression of *Nrf2* in human beings.²⁰

Given that conjugation of reduced glutathione with the oxidative metabolite of acetaminophen, NAPQI, is partly catalyzed by *GSTs*, we hypothesized that the effects of prenatal acetaminophen might also be modified by common polymorphisms in the *GSTs*. However, this is highly speculative, because we are not aware of any evidence to suggest that the common *GST* polymorphisms influence acetaminophen toxicity in human beings. Furthermore, despite *in vitro* evidence indicating that *GSTP1* is a particularly effective catalyst of the conjugation of glutathione with NAPQI,²⁸ experiments in mice lacking *GSTP1* showed that this is not the case *in vivo*; unexpectedly, these mice were resistant to acetaminophen hepatotoxicity,¹⁴ as well as having much heavier lungs compared with control mice.²⁹ In contrast with a recent, and much smaller, birth cohort study from the United States in which Perzanowski et al⁵ reported an interaction between child *GSTP1* genotype and prenatal acetaminophen exposure on risk of childhood wheezing, we did not find any evidence for modification of the effects of prenatal acetaminophen on asthma and wheezing by maternal or child *GSTP1*. However, in keeping with the interactions we observed with maternal and child *GSTM1* and *GSTT1* genotypes in our data, Perzanowski et al⁵ also reported increased risks of wheezing associated with prenatal acetaminophen in the presence of *GSTM1* and *GSTT1* genotypes in the children, although the interactions in their study were not significant, and

they did not comment on these counterintuitive observations. These findings are reminiscent of the surprising results in *GSTP1* knockout mice. In that model, it was reported that greater depletion of glutathione was observed in wild-type mice than in null mice, presumably because the former were able to conjugate glutathione with NAPQI more efficiently.¹⁴ We therefore propose that mothers and children who are homozygous wild for *GSTM1/T1* are more likely than those with null genotypes to deplete glutathione when exposed to acetaminophen. We found some evidence to suggest that the increased risk of asthma and wheezing associated with late gestation acetaminophen exposure in the presence of maternal *GSTM1* genotype was further enhanced if *GSTM1* was also present in the child. Acetaminophen crosses the placenta,³⁰ and the fetus is capable of generating the toxic metabolite NAPQI in late gestation, but to a lesser degree before 20 weeks.³¹ Interestingly, in animals, pregnancy is associated with increased hepatotoxicity of acetaminophen, and this effect is associated with greater depletion of hepatic glutathione.³²

Two observations suggest that therapeutic doses of acetaminophen may have important effects on oxidant/antioxidant balance in human beings, even when used in doses that would not be expected to cause hepatotoxicity. First, *in vitro*, acetaminophen reduces intracellular glutathione levels in human alveolar macrophages and type II pneumocytes³³; second, chronic ingestion of maximum therapeutic doses of acetaminophen can reduce serum antioxidant capacity in a few weeks.³⁴

Effects of infant acetaminophen exposure

In keeping with our previous report of an association between infant acetaminophen use and early persistent wheezing,⁶ we have confirmed a longitudinal association with childhood asthma that was independent of prenatal acetaminophen exposure. However, this relation was limited to children who had wheezed in infancy. The most likely explanation is that infants who already have a wheezing tendency are more likely to be given acetaminophen for viral respiratory infections with fever. Similarly, the association with atopy may reflect a tendency for infants who are already atopic to have more severe infections and consequently to be given acetaminophen. Although we cannot rule out the possibility that infant acetaminophen exposure might contribute to

TABLE II. Associations between maternal use of acetaminophen in early and late pregnancy and children's asthma stratified by maternal genotype

Genotype	Maternal acetaminophen use							
	Early pregnancy				Late pregnancy			
	N	OR*	(95% CI)	P value	N	OR*	(95% CI)	P value
<i>Nrf2</i>								
C:C	3754	0.99	(0.81, 1.21)	.91	3651	1.19	(0.96, 1.46)	.11
T:C/T:T	1137	1.73	(1.22, 2.45)	.002	1114	1.63	(1.13, 2.37)	.009
Interaction	4891			.02	4765			.18
<i>GSTT1</i>								
Copy number								
2	1223	1.30	(0.92, 1.83)	.13	1178	1.46	(1.00, 2.15)	.05
1	1952	1.24	(0.94, 1.63)	.12	1910	1.36	(1.04, 1.78)	.03
0	774	0.89	(0.57, 1.38)	.6	758	0.75	(0.46, 1.22)	.24
Interaction	3949			.3	3846			.03
Present	3838	1.24	(1.02, 1.50)	.03	3730	1.39	(1.14, 1.70)	.001
Absent	774	0.89	(0.57, 1.38)	.6	758	0.75	(0.46, 1.22)	.24
Interaction	4612			.14	4488			.006
<i>GSTM1</i>								
Copy number								
2	307	1.96	(1.09, 3.51)	.03	296	2.04	(0.96, 4.31)	.06
1	1705	1.00	(0.73, 1.36)	.98	1670	1.41	(1.02, 1.95)	.04
0	2462	1.27	(1.00, 1.62)	.05	2389	1.14	(0.89, 1.46)	.31
Interaction	4474			.18	4355			.35
Present	2188	1.11	(0.86, 1.44)	.44	2137	1.45	(1.10, 1.90)	.009
Absent	2462	1.27	(1.00, 1.62)	.05	2389	1.14	(0.89, 1.46)	.31
Interaction	4650			.44	4526			.22
<i>GSTP1</i>								
A:A	2059	1.28	(0.96, 1.70)	.09	2003	1.11	(0.82, 1.49)	.49
G:A	2240	1.04	(0.81, 1.33)	.78	2180	1.25	(0.95, 1.64)	.11
G:G	593	1.37	(0.88, 2.15)	.16	577	1.40	(0.89, 2.20)	.14
Interaction	4892			.43	4760			.68

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, antibiotic use and alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7 years, infant acetaminophen use).

the maintenance of asthma symptoms or the inception of atopy, the lack of interaction between this exposure and antioxidant gene polymorphisms would argue against a causal interpretation. There is a paucity of data on the relation between acetaminophen exposure in early life and atopy, measured objectively by skin testing or IgE measurement. Exposure in children and adults has been linked to cockroach, but not dust mite, sensitization in an Ethiopian study,³⁵ and we found only weak evidence for an ecologic relation in adults.² Perzanowski et al⁵ reported a relation between prenatal exposure and seroatopy that was modified by child *GSTT1* genotype. In contrast, we did not observe an overall relation between prenatal exposure and atopy measured by skin prick testing, although we did find a significant interaction with maternal (but not child) *GSTT1* genotype. It is possible (in addition to differences in sample size) that ethnic differences between ALSPAC (largely white) and the US cohort study (Dominican Republic and African American) may have contributed to conflicting findings. For example, the *GSTM1* deletion was more than twice as common in ALSPAC.

Strengths and limitations

The ALSPAC has a number of strengths, aside from its population-based prospective design and data on prenatal and postnatal acetaminophen exposure. First, ALSPAC's size gives us

greater power to detect gene-environment interactions than can be achieved in smaller birth cohorts. Second, few other birth cohorts have had maternal DNA available to explore interactions with prenatal exposures; a disadvantage of having only child genotype is that this could modify both prenatal and postnatal exposures, and these exposures may confound each other; in contrast, maternal genotype cannot directly influence postnatal acetaminophen exposure. Third, we determined *GSTM1* and *GSTT1* genotypes in more detail than previous studies; by measuring copy number variation, we observed the greatest effects of prenatal acetaminophen on asthma in the presence of homozygous wild genotypes (defining genotype by presence or absence of the null deletion combines homozygous wild with hemizygotes). Fourth, unlike in previous studies,⁴ we had prospectively collected data on infant wheezing, which enabled us to show clearly that the link between infant exposure and later asthma was limited to those who had wheezed in infancy. A final strength is the detailed phenotypic measurements. A recent report suggested that late gestation acetaminophen exposure might be associated with increased neonatal BR; however, the authors acknowledged that this finding was probably influenced by extreme outlier values.³⁶ In our much larger cohort, we found no relation with bronchial responsiveness or lung function measured later in childhood. Although we carried out multiple statistical comparisons, we were testing an *a priori* hypothesis with respect to specific antioxidant genes, and

TABLE III. Associations between maternal use of acetaminophen in early and late pregnancy and children's wheezing stratified by maternal genotype

Maternal genotype		Maternal acetaminophen use						
Genotype	Early pregnancy			P value	Late pregnancy			P value
	N	OR*	(95% CI)		N	OR*	(95% CI)	
<i>Nrf2</i>								
C:C	3800	0.90	(0.73, 1.12)	.35	3696	1.13	(0.91, 1.40)	.28
T:C/T:T	1149	1.53	(1.06, 2.20)	.024	1126	1.42	(0.96, 2.09)	.08
Interaction	4949			.04	4822			.35
<i>GSTT1</i>								
Copy number								
2	1235	1.06	(0.75, 1.52)	.73	1191	1.39	(0.95, 2.05)	.09
1	1977	1.14	(0.84, 1.54)	.41	1934	1.16	(0.86, 1.55)	.34
0	786	0.77	(0.48, 1.23)	.28	770	0.82	(0.49, 1.38)	.46
Interaction	3998			.33	3895			.24
Present	3883	1.12	(0.91, 1.38)	.29	3775	1.27	(1.03, 1.56)	.03
Absent	786	0.77	(0.48, 1.23)	.28	770	0.82	(0.49, 1.38)	.46
Interaction	4669			.11	4545			.08
<i>GSTM1</i>								
Copy number								
2	313	1.55	(0.83, 2.89)	.17	303	2.15	(0.94, 4.88)	.07
1	1727	1.07	(0.77, 1.49)	.68	1692	1.60	(1.15, 2.24)	.006
0	2491	1.03	(0.79, 1.33)	.85	2417	0.98	(0.75, 1.28)	.88
Interaction	4531			.6	4412			.06
Present	2217	1.10	(0.84, 1.45)	.49	2167	1.50	(1.13, 2.01)	.006
Absent	2491	1.03	(0.79, 1.33)	.85	2417	0.98	(0.75, 1.28)	.88
Interaction	4708			.71	4584			.04
<i>GSTP1</i>								
A:A	2085	1.21	(0.90, 1.62)	.21	2029	1.15	(0.85, 1.55)	.36
G:A	2266	0.93	(0.70, 1.23)	.61	2205	1.14	(0.85, 1.53)	.37
G:G	601	1.15	(0.71, 1.87)	.57	585	1.11	(0.68, 1.82)	.68
Interaction	4952			.43	4819			.99

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, antibiotic use and alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7 years, infant acetaminophen use).

TABLE IV. Unadjusted and adjusted effects of infant acetaminophen use on childhood asthma and related phenotypes

Outcome	N	Unadjusted	(95% CI)	P value	Adjusted*	(95% CI)	P value
OR							
Asthma	7735	1.19	(1.08, 1.32)	<.001	1.11	(1.00, 1.23)	.046
Wheezing	7821	1.20	(1.08, 1.34)	<.001	1.12	(1.00, 1.25)	.045
Eczema	7809	1.09	(1.00, 1.18)	.06	1.05	(0.97, 1.15)	.23
Atopy	6092	1.15	(1.05, 1.26)	.002	1.14	(1.04, 1.25)	.005
Geometric mean ratio							
BHR slope	4118	1.07	(1.00, 1.15)	.06	1.04	(0.97, 1.12)	.3
Total IgE	4812	0.99	(0.93, 1.06)	.88	0.97	(0.90, 1.03)	.32
Arithmetic mean difference (SD units)							
Adjusted FVC	6327	-0.01	(-0.04, 0.03)	.7	-0.01	(-0.04, 0.03)	.69
Adjusted FEV ₁	6231	0.00	(-0.03, 0.04)	.8	0.01	(-0.03, 0.04)	.66
Adjusted FEF ₂₅₋₇₅	6327	0.00	(-0.03, 0.04)	.81	0.01	(-0.03, 0.04)	.69

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, acetaminophen and antibiotic use, alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7 years).

although we explored possible interactions with various phenotypic outcomes, our primary outcomes of interest were asthma and wheezing, because prenatal acetaminophen had the strongest effects on these outcomes overall.

In considering potential limitations of our study, we should first consider the main effects of prenatal acetaminophen exposure. As

with any longitudinal study, data were not complete on exposures, outcomes, and confounders for the whole cohort. Therefore, we cannot rule out the possibility that exclusion of children without complete information might have biased our findings. However, for the adverse effects of prenatal acetaminophen on asthma wheezing to be spurious, there would have to be a protective effect

TABLE V. Adjusted effects of infant acetaminophen use on childhood asthma and related phenotypes according to wheezing in the first 6 months

Outcome	No wheeze				P value	Wheeze				P value
	N	OR*	(95% CI)			N	OR*	(95% CI)		
Asthma	6293	1.03	(0.92, 1.16)	.63		1392	1.44	(1.13, 1.83)	.003	
Wheezing	6356	1.07	(0.94, 1.22)	.31		1412	1.23	(0.98, 1.55)	.08	
Eczema	6350	1.02	(0.93, 1.12)	.65		1406	1.21	(0.96, 1.52)	.1	
Atopy	4907	1.14	(1.03, 1.27)	.009		1143	1.08	(0.85, 1.38)	.54	

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, acetaminophen and antibiotic use, alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7 years).

of equal magnitude in those children who were not included, which seems unlikely, especially because our findings have been replicated in other cohorts. Although a strength of the information on exposure is that it was collected during pregnancy, a limitation is the limited number of categories of frequency of use. However, such misclassification is likely to have been random with respect to outcomes, which would tend to lead to an underestimation of effects. Although we used sensitivity to only 3 aeroallergens to define atopy, we have previously shown that this definition identified 96% of children sensitized to 26 other allergens in this cohort,³⁷ and therefore any misclassification of atopy will have been minor. We believe that the effects of prenatal acetaminophen on asthma and wheezing are unlikely to be confounded for a number of reasons. First, we controlled for an extensive number of potential confounders by using a propensity score approach. Confounding by indication is of potential concern, because there were no data on indications for acetaminophen use during pregnancy or infancy. The major indication for acetaminophen use in infancy is likely to be febrile infections. However, the most common reasons during pregnancy are likely to be for headache and musculoskeletal complaints, and we have no reason to believe musculoskeletal disorders would confound relations with childhood asthma. Some mothers may have taken acetaminophen for migraine, and there is some evidence that asthma may be more common in the offspring of mothers with migraine,³⁸ but we controlled for a history of maternal migraine in the analyses. We also controlled for infections and antibiotic use in pregnancy. Finally, we have recently reported evidence in this cohort to suggest that the relation between maternal use of acetaminophen in pregnancy and childhood asthma is unlikely to be confounded by unmeasured behavioral factors linked to acetaminophen use.³⁹ With respect to the main findings of this study, namely the interactions between maternal genotype and prenatal acetaminophen exposure, although the genotypes were selected *a priori*, we acknowledge that some of these apparent interactions may have arisen by chance, and therefore our findings require replication in other birth cohort studies of adequate size.

In conclusion, we have found evidence to suggest interactions between prenatal acetaminophen exposure and maternal antioxidant genotypes on childhood asthma risk. This increases the likelihood that the acetaminophen-asthma link is causal. To

TABLE VI. Associations between infant use of acetaminophen and childhood asthma stratified by child genotype

Genotype	N	OR*	(95% CI)	P value
<i>GSTT1</i>				
Copy number				
2	1640	0.96	(0.78, 1.17)	.66
1	2411	1.07	(0.89, 1.29)	.48
0	977	1.13	(0.84, 1.53)	.42
Interaction	5028			.59
Present	4756	1.03	(0.91, 1.17)	.65
Absent	977	1.13	(0.84, 1.53)	.42
Interaction	5733			.58
<i>GSTM1</i>				
Copy number				
2	382	0.86	(0.49, 1.48)	.58
1	2074	1.08	(0.89, 1.32)	.45
0	3082	1.02	(0.87, 1.20)	.81
Interaction	5538			.69
Present	2682	1.11	(0.93, 1.33)	.25
Absent	3082	1.02	(0.87, 1.20)	.81
Interaction	5764			.49
<i>GSTP1</i>				
A:A	2467	0.98	(0.82, 1.17)	.8
G:A	2592	1.14	(0.96, 1.35)	.14
G:G	719	1.12	(0.76, 1.65)	.57
Interaction	5778			.47
<i>Nrf2</i>				
C:C	4402	1.10	(0.97, 1.26)	.14
T:C/T:T	1353	0.93	(0.72, 1.20)	.58
Interaction	5755			.22

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, acetaminophen and antibiotic use, alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7 years).

confirm this, experimental studies in animal models could provide supportive evidence, although definitive evidence in human beings can come only from randomized controlled trials,⁴⁰ which will be challenging to carry out.

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

Clinical implications: Further evidence suggesting possible causal links between acetaminophen use in pregnancy and infancy and childhood asthma is important given the high prevalence of both acetaminophen use and asthma in western populations.

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METHODS

Further details on lung function and bronchial responsiveness measurements

Spirometry. The tests adhered to American Thoracic Society (ATS) criteria for standardisation and reproducibility of flow-volume measurement,^{E1} with the exception of ATS recommendations for duration of expiration^{E2}; as many children did not fulfil forced expiratory time >6 seconds end of test criteria, a minimal volume change over the final 1 second was used.

Bronchial responsiveness. Saline (0.9%) solution was administered and a postsaline FEV₁ measurement was used as the baseline. Subsequently, 8 doubling doses of methacholine from 0.05 to 6.1 μ mol were given at 1-minute intervals with repeat measurement of FEV₁ after each dose. The challenge continued until the FEV₁ decreased by $\geq 20\%$ from baseline or the maximum dose of methacholine had been given. The FEV₁ following each dose of methacholine was expressed as a percent of baseline FEV₁, and a linear regression (dose-response) slope of relative FEV₁ with respect to cumulative

methacholine dose was calculated for each subject and expressed in percent per micromole of methacholine. To prevent the analysis from being dominated by extreme positive and negative slopes based on small numbers of observations, we recoded all negative slopes to 0%/micromole, and all slopes above 50%/micromole to 50%/micromole, to derive a truncated slope, and then added 0.1%/micromole to these truncated slopes to derive a transformed slope, whose geometric means (GMs) and GM ratios were estimated in the statistical analysis. A higher GM denotes a greater level of bronchial responsiveness.

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TABLE E1. Confounders used to generate acetaminophen propensity scores

Confounder	Values
Male child	No, Yes
Mother's age (y)	<20, 20-24, 25-29, 30-34, 35+
Prenatal tobacco exposure (maximum in pregnancy)	Not exposed, Passive only, Mother 1-9/d, Mother 10-19/d, Mother 20+/d, Unknown
Mother's education level	<Ordinary level, Ordinary level, Advanced level +, Unknown
Housing tenure	Owned/mortgaged, Rented (public housing), Rented (non-public housing), Unknown/other
Parity	0, 1, 2+, Unknown
Maternal anxiety score	0-4, 5-9, 10+, Unknown
Mother's ethnic origin	White, Nonwhite, Unknown
Maternal BMI (kg/m ²)	<18.5, 18.5-24.99, 25-29.99, 30+, Unknown
Season of birth	Jan-Mar, Apr-Jun, Jul-Sep, Oct-Dec
Multiple pregnancy	No, Yes
Antibiotics at 20-32 wk gestation	Not reported, Reported
Alcohol units/wk at 0-8 wk gestation	Never, 1-2/wk, 3-4/wk, 5+/wk, Unknown
Alcohol glasses/wk at 0-18 wk gestation	Never, <1/wk, 1-6/wk, 1+/d, Unknown
Alcohol units/wk at 18-32 wk gestation	Never, 1-2/wk, 3+/wk, Unknown
Alcohol units/wk over last 2 mo gestation	Never, <1/wk, 1+/wk, Unknown
Financial difficulties	None, Some, Many, Unknown
Maternal disease history unknown	No, Yes
Maternal asthma history	No, Yes
Maternal eczema history	No, Yes
Maternal rhinoconjunctivitis history	No, Yes
Maternal migraine history	No, Yes
Maternal infection history unknown	No, Yes
Maternal cold/flu history	No, Yes
Maternal urinary infection history	No, Yes
Maternal other infections history	No, Yes
Younger siblings at 7 y	None, 1, 2+, Unknown
Pets in first year	None, Cat or dog, Other pet, Unknown
Breast-fed in first 6 mo	No, Yes, Unknown
Child in day care at 8 mo	No, Yes, Unknown
Damp, condensation, and mold	No, Yes, Unknown
Child exposed to tobacco smoke at weekend	No, Yes, Unknown
Gestational age (wk)	<37, 37-40, 41+
Birth weight (g)	<2500, 2500-2999, 3000-3499, 3500-3999, 4000+, Unknown
Birth length (cm)	<48, 48-50.99, 51-53.99, 54+, Unknown
Head circumference (cm)	<33, 33-34.99, 35-36.99, 37+, Unknown
Child BMI at 7 y	<15.00, 15.00-17.49, 17.50-20.49, 20.5+, Unknown

TABLE E2. Genotyping failure and error rates

Genotyping failure rate	Child	Mother
<i>Nrf2</i>	6.0%	4.9%
<i>GSTP1</i>	9.0%	4.0%
<i>GSTT1</i> (gene present/absent)	5.7%	4.1%
<i>GSTT1</i> (copy number)	17.6%	17.2%
<i>GSTM1</i> (gene present/absent)	5.4%	3.8%
<i>GSTM1</i> (copy number)	9.2%	7.5%
Genotyping error rate	Child	Mother
<i>Nrf2</i>	<0.22%	<0.08%
<i>GSTP1</i>	0.22%	0.64%
<i>GSTT1</i> (gene present/absent)	0.22%	0.09%
<i>GSTT1</i> (copy number)	1.67%	1.80%
<i>GSTM1</i> (gene present/absent)	0.45%	0.09%
<i>GSTM1</i> (copy number)	0.73%	0.09%

Percentages refer to the genotyping failure and error rates for the different gene assays (copy number or present/absent).

TABLE E3. Associations between maternal use of acetaminophen in early and late pregnancy and children's asthma stratified by child's genotype

Genotype	Maternal acetaminophen use							
	Early pregnancy				Late pregnancy			
	N	OR*	(95% CI)	P value	N	OR*	(95% CI)	P value
<i>Nrf2</i>								
C:C	4527	1.22	(1.02, 1.46)	.03	4379	1.34	(1.11, 1.61)	.002
T:C/T:T	1374	1.24	(0.89, 1.73)	.21	1345	1.24	(0.87, 1.76)	.24
Interaction	5901			.94	5724			.7
<i>GSTT1</i>								
Copy number								
2	1682	1.11	(0.82, 1.51)	.51	1635	1.33	(0.96, 1.83)	.08
1	2478	1.31	(1.03, 1.66)	.03	2404	1.24	(0.97, 1.59)	.09
0	1001	1.05	(0.71, 1.55)	.82	970	1.46	(0.96, 2.22)	.08
Interaction	5161			.55	5009			.81
Present	4876	1.25	(1.05, 1.49)	.01	4736	1.28	(1.07, 1.53)	.008
Absent	1001	1.05	(0.71, 1.55)	.82	970	1.46	(0.96, 2.22)	.08
Interaction	5877			.39	5706			.58
<i>GSTM1</i>								
Copy number								
2	405	2.72	(1.45, 5.11)	.002	398	4.18	(1.90, 9.20)	<.001
1	2137	0.91	(0.70, 1.19)	.49	2076	1.16	(0.87, 1.53)	.31
0	3140	1.22	(0.98, 1.52)	.08	3035	1.12	(0.89, 1.41)	.34
Interaction	5682			.04	5509			.19
Present	2772	1.19	(0.95, 1.50)	.14	2701	1.44	(1.14, 1.83)	.003
Absent	3140	1.22	(0.98, 1.52)	.08	3035	1.12	(0.89, 1.41)	.34
Interaction	5912			.88	5736			.14
<i>GSTP1</i>								
A:A	2524	1.38	(1.07, 1.79)	.01	2442	1.40	(1.08, 1.82)	.01
G:A	2654	1.06	(0.85, 1.32)	.61	2584	1.13	(0.89, 1.44)	.31
G:G	736	0.99	(0.60, 1.61)	.95	713	1.69	(1.02, 2.79)	.04
Interaction	5914			.27	5739			.3

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, antibiotic use and alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7 years, infant acetaminophen use).

TABLE E4. Associations between maternal use of acetaminophen in early and late pregnancy and children's wheezing stratified by child's genotype

Genotype	Maternal acetaminophen use							
	Early pregnancy				Late pregnancy			
	N	OR*	(95% CI)	P value	N	OR*	(95% CI)	P value
<i>Nrf2</i>								
C:C	4576	1.17	(0.96, 1.42)	.11	4427	1.23	(1.01, 1.49)	.04
T:C/T:T	1391	1.06	(0.75, 1.50)	.73	1359	1.09	(0.76, 1.58)	.64
Interaction	5967			.62	5786			.56
<i>GSTT1</i>								
Copy number								
2	1705	1.04	(0.75, 1.44)	.83	1656	0.95	(0.68, 1.32)	.77
1	2502	1.19	(0.93, 1.53)	.16	2426	1.34	(1.03, 1.73)	.03
0	1015	0.91	(0.61, 1.36)	.64	984	1.18	(0.78, 1.79)	.43
Interaction	5222			.49	5066			.26
Present	4930	1.17	(0.97, 1.41)	.1	4786	1.17	(0.97, 1.41)	.1
Absent	1015	0.91	(0.61, 1.36)	.64	984	1.18	(0.78, 1.79)	.43
Interaction	5945			.24	5770			.96
<i>GSTM1</i>								
Copy number								
2	414	2.52	(1.17, 5.43)	.02	406	4.77	(2.12, 10.75)	<.001
1	2155	0.86	(0.65, 1.15)	.32	2094	0.99	(0.74, 1.32)	.94
0	3179	1.12	(0.89, 1.42)	.33	3071	1.06	(0.84, 1.34)	.64
Interaction	5748			.11	5571			.16
Present	2801	1.14	(0.90, 1.46)	.28	2729	1.31	(1.02, 1.68)	.03
Absent	3179	1.12	(0.89, 1.42)	.33	3071	1.06	(0.84, 1.34)	.64
Interaction	5980			.91	5800			.23
<i>GSTP1</i>								
A:A	2554	1.20	(0.92, 1.58)	.18	2470	1.27	(0.97, 1.65)	.08
G:A	2687	1.13	(0.90, 1.43)	.29	2616	1.12	(0.88, 1.43)	.37
G:G	743	0.73	(0.43, 1.26)	.26	719	1.41	(0.81, 2.46)	.23
Interaction	5984			.16	5805			.68

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, antibiotic use and alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7 years, infant acetaminophen use).

TABLE E5. Associations between infant use of acetaminophen and childhood wheezing stratified by child's genotype

Genotype	N	OR*	(95% CI)	P value
<i>GSTT1</i>				
Copy number				
2	1661	0.99	(0.79, 1.24)	.91
1	2434	1.02	(0.84, 1.23)	.87
0	991	1.42	(1.02, 1.96)	.04
Interaction	5086			.24
Present	4807	0.99	(0.86, 1.13)	.83
Absent	991	1.42	(1.02, 1.96)	.04
Interaction	5798			.08
<i>GSTM1</i>				
Copy number				
2	391	0.67	(0.40, 1.13)	.14
1	2090	1.13	(0.91, 1.41)	.27
0	3120	0.99	(0.84, 1.17)	.88
Interaction	5601			.11
Present	2709	1.14	(0.94, 1.38)	.19
Absent	3120	0.99	(0.84, 1.17)	.88
Interaction	5829			.29
<i>GSTP1</i>				
A:A	2496	0.97	(0.80, 1.18)	.79
G:A	2624	1.11	(0.93, 1.32)	.25
G:G	725	1.40	(0.87, 2.26)	.17
Interaction	5845			.37
<i>Nrf2</i>				
C:C	4449	1.09	(0.95, 1.26)	.23
T:C/T:T	1369	0.94	(0.72, 1.23)	.67
Interaction	5818			.33

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, acetaminophen and antibiotic use, alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7 years).

TABLE E6. Associations between infant use of acetaminophen and childhood atopy stratified by child's genotype

Genotype	N	OR*	(95% CI)	P value
<i>GSTT1</i>				
Copy number				
2	1414	0.99	(0.81, 1.20)	.9
1	2124	1.08	(0.93, 1.27)	.32
0	831	1.25	(0.96, 1.63)	.1
Interaction	4369			.4
Present	4156	1.09	(0.98, 1.23)	.12
Absent	831	1.25	(0.96, 1.63)	.1
Interaction	4987			.39
<i>GSTM1</i>				
Copy number				
2	342	1.54	(1.02, 2.32)	.04
1	1778	1.06	(0.89, 1.25)	.53
0	2680	1.12	(0.96, 1.29)	.14
Interaction	4800			.34
Present	2319	1.13	(0.97, 1.30)	.12
Absent	2680	1.12	(0.96, 1.29)	.14
Interaction	4999			.94
<i>GSTP1</i>				
A:A	2131	1.16	(0.99, 1.35)	.07
G:A	2271	1.06	(0.92, 1.23)	.43
G:G	628	1.43	(1.01, 2.01)	.042
Interaction	5030			.34
<i>Nrf2</i>				
C:C	3813	1.10	(0.98, 1.24)	.11
T:C/T:T	1176	1.20	(0.96, 1.50)	.1
Interaction	4989			.51

*Adjusted for maternal factors in pregnancy (smoking, infections, anxiety score, acetaminophen and antibiotic use, alcohol intake), other maternal factors (educational level, housing tenure, financial difficulties, BMI, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine), sex of child, season of birth, multiple pregnancy, gestational age, birth weight, head circumference, birth length, and postnatal factors (breast-feeding, day care, pets, damp/mold, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7 years).