Published in final edited form as:

J Allergy Clin Immunol. 2010 December ; 126(6): 1141–8.e7. doi:10.1016/j.jaci.2010.08.047.

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

Seif O Shaheen, PhD¹, Roger B Newson, DPhil¹, Susan M Ring, PhD², Matthew J Rose-Zerilli, BSc³, John W Holloway, PhD³, and A John Henderson, MD⁴

¹Respiratory Epidemiology & Public Health Group, National Heart and Lung Institute, Imperial College London

²Department of Social Medicine, University of Bristol

³Respiratory Genetics Group, Division of Infection, Inflammation & Immunity and Division of Human Genetics, University of Southampton

⁴Department of Community-based Medicine, University of Bristol

Abstract

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 (ALSPAC) 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 >4,000 mothers and >5,000 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 interaction 0.02 and 0.04, respectively). Risk of asthma associated with late gestation exposure was higher when maternal GSTT1 genotype was present, rather than absent (P interaction 0.006), and risk of wheezing was increased when maternal GSTM1 was present (P interaction 0.04). Whilst acetaminophen use in infancy was associated with an increased risk of atopy, child antioxidant

Correspondence and reprint requests to: Prof Seif Shaheen, 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, UK., s.shaheen@qmul.ac.uk, Tel: +44 (0)20 7882 2480; Fax: +44 (0)20 7882 2552.

Contributors

All authors contributed to study design. MJR-Z, SMR, and JWH were responsible for collection of genetic data. AJH was responsible for all respiratory and allergy phenotype data collection. SOS and RBN analysed the data. SOS wrote the first draft of the manuscript, and all authors have seen and approved the final version of the report. SOS and AJH will serve as guarantors for its contents.

Conflict of interest statement

None of the authors have any conflicts of interests to declare.

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.

Keywords

Asthma; Acetaminophen; paracetamol; Glutathione-S-transferase; Nrf2; Prenatal Exposure; Delayed Effects; ALSPAC; pregnancy; birth cohort; genotype; gene environment interaction

Introduction

Following our original observations in adults and children^{1,2}, a large body of epidemiological evidence has accumulated linking acetaminophen (paracetamol) exposure throughout the lifecourse to asthma³. However, the majority of studies which 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 based on recall by mothers five years later⁴. 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 five years in a small US birth cohort⁵. 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 atopy⁷. 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 Cohort⁸, and two small US studies have recently confirmed an association with wheezing in infancy⁹ and in childhood⁵, 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 of glutathione (GSH)^{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 expression¹⁰. Furthermore, Nrf2 knock-out mice are particularly sensitive to acetaminophen

hepatotoxicity^{11–13}. The GSTs also play a role in detoxification of the oxidative metabolite of acetaminophen, NAPQI, through conjugation with GSH, and GSTP1 has been shown to influence acetaminophen hepatotoxicity in knock-out mice¹⁴.

Previous cross-sectional studies of infant acetaminophen exposure have not had information on prenatal exposure, in order 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. In order 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 Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort that recruited 14,541 pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. There were 14,062 live born children and 13,988 of these children were alive at age one 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 previously¹⁵ 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 18–20 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 three months. As 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 six 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; hayfever?’. 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 one 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 *D. pteronyssinus*, cat or grass (after subtracting positive saline reactions from histamine and allergen weals, and excluding children unreactive to 1% histamine). This was

used to define atopic and non-atopic asthma. Serum total IgE (kU/l) was measured by fluoroimmunoassay using the Pharmacia UNICAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Lung function was measured by spirometry (Vitalograph 2120) at age 8½ 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 three reproducible flow-volume curves was used to measure FEV₁, FVC and maximal mid expiratory flow (FEF₂₅₋₇₅). Lung function measurements were transformed to age, height and gender adjusted standard deviation units¹⁶. Bronchial responsiveness (BR) to methacholine was measured using the method of Yan¹⁷. BR was expressed as the dose-response slope (% FEV decline/μmole methacholine). (See online repository for further details).

Confounders

For each acetaminophen exposure (early and late gestation and infancy) we defined propensity scores¹⁸, 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, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7). 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 six months after birth. Details of confounder categories are given in Table E1 of the online repository materials. 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 non-convergence 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, with a small number extracted from venous blood collected at 43-61 months. Two single nucleotide polymorphisms (SNPs) were typed by KBiosciences Ltd (Hoddesdon, Herts, UK; 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 individuals²⁰. In our data the Nrf2 SNP was typed using the reverse strand (C/T). The GSTT1 & GSTM1 gene deletion genotyping was performed in Southampton (MJR-Z) using a real-time PCR method described previously²¹. 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 Table E2 (online repository).

Statistical analyses

Acetaminophen exposures were analysed as linear per category effects 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 using Huber variances²². The confounder-adjusted effects of acetaminophen were stratified by maternal and child GST genotype. To test for gene-acetaminophen interaction we used a Wald chi-squared or F-test for heterogeneity between the acetaminophen effects in the genotype strata. As 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

Out 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 1 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 exposure was 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. Whilst 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 two exposures separate. If we omitted variables from the model which could theoretically be on the causal pathway (gestational age²³, birth anthropometry and child's BMI at age 7), and therefore should not be controlled for²⁴, the effects of prenatal acetaminophen on asthma and wheezing were even stronger (data not shown). The associations with asthma were limited to the non-atopic phenotype; no association was seen with atopic asthma (data not shown). There were no associations with lung function or bronchial responsiveness (BR).

Table 2 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 one or two copies of the minor T allele of the Nrf2 promoter SNP. This was particularly evident for early gestation exposure (P interaction 0.02). There was also evidence of effect modification by GSTT1, especially for late gestation exposure, with increased risk of asthma occurring in the presence of one or two copies, but not when

GSTT1 was null (P interaction for null genotype 0.006). Similarly, risk was increased in the presence of two copies of GSTM1. There was no convincing evidence for modification by maternal GSTP1.

Table 3 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 0.04). There was also evidence of modification of the effect of late gestation exposure on wheezing by GSTT1 and GSTM1 null genotypes (P interaction 0.08 and 0.04, respectively). There was no evidence of interaction between maternal GSTP1 genotype and prenatal acetaminophen on wheezing.

Tables E3 and E4 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 two 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 for asthma (n=1103) 1.73 (95% CI: 1.17 to 2.55), P=0.006; OR for wheeze (n=1119) 1.67 (1.12 to 2.49), P=0.01). 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 0.02 and 0.003, respectively), with increased risk when GSTT1 was present (OR for late gestation exposure 1.19 (0.99 to 1.42), n=2969), and reduced risk when the genotype was null (OR 0.64 (0.41 to 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 0.03). There were no interactions between maternal or child genotype and prenatal acetaminophen exposure on total IgE.

Out of 11,438 infants with data on infant acetaminophen use, 14% were not given the drug in the first six months after birth, 20% were given it once, and 66% were given it on two or more occasions. Table 4 shows the unadjusted and adjusted effects of acetaminophen use in the first six 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 (odds ratio 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 later 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 six months, we found that the risks of wheezing, eczema, and especially asthma, but not atopy, were greater in children who wheezed in infancy (Table 5). Table 6 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 (Tables E5 and E6).

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

Nrf2 was of *a priori* interest for two reasons. First, because knock-out of this gene in mice increases susceptibility to acetaminophen hepatotoxicity^{11–13}. Second, because of its central role in induction of antioxidant gene transcription, defending the lung against oxidative stress¹⁰, and protecting against pulmonary disease²⁵. Disruption of Nrf2 leads to severe allergen-driven airway inflammation, hyper-responsiveness, and increased Th2 cytokine levels in a mouse model of asthma²⁶ and, in humans, polymorphisms in Nrf2 have recently been linked to adult lung function²⁷ and to the risk of acute lung injury²⁰. 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 defences are compromised; the minor allele has been associated with reduced expression of Nrf2 in humans²⁰.

Given that conjugation of reduced glutathione (GSH) with the oxidative metabolite of acetaminophen, NAPQI, is partly catalysed by GSTs, we hypothesised that the effects of prenatal acetaminophen might also be modified by common polymorphisms in the GSTs. However, this is highly speculative, as we are not aware of any evidence to suggest that the common GST polymorphisms influence acetaminophen toxicity in humans. Furthermore, despite *in vitro* evidence indicating that GSTP1 is a particularly effective catalyst of the conjugation of GSH with NAPQI²⁸, experiments in mice lacking GSTP1 showed that this is not the case *in vivo*; unexpectedly, these mice were *resistant* to acetaminophen hepatotoxicity¹⁴, as well as having much heavier lungs compared to control mice²⁹. In contrast to a recent, and much smaller, birth cohort study from the USA in which Perzanowski et al reported an interaction between child GSTP1 genotype and prenatal acetaminophen exposure on risk of childhood wheezing⁵, 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 and colleagues 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 GSH was observed in wild-type mice than in null mice, presumably because the former were able to conjugate GSH with NAPQI more efficiently¹⁴. We therefore propose that mothers and children who are homozygous wild for GSTM1/T1 are more likely than those with null genotypes to deplete GSH 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 placenta³⁰, and the fetus is capable of generating the toxic metabolite NAPQI in late gestation, but to a lesser degree before 20 weeks³¹. Interestingly, in animals, pregnancy is associated with increased hepato-toxicity of acetaminophen, and this effect is associated with greater depletion of hepatic glutathione³².

Two observations suggest that therapeutic doses of acetaminophen may have important effects on oxidant/antioxidant balance in humans, even when used in doses which would not be expected to cause hepatotoxicity. First, in vitro, acetaminophen reduces intracellular glutathione (GSH) 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 weeks³⁴.

Effects of infant acetaminophen exposure

In keeping with our previous report of an association between infant acetaminophen use and early persistent wheezing⁶, we have confirmed a longitudinal association with childhood asthma, which 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 to consequently be given acetaminophen. Whilst we cannot rule out the possibility that infant acetaminophen exposure might contribute to 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, sensitisation in an Ethiopian study³⁵, and we only found weak evidence for an ecological relation in adults². Perzanowski and colleagues reported a relation between prenatal exposure and seroatopy⁵, which 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 Caucasian) 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

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 only having 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 previous studies⁴, 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 BHR; however, the authors acknowledged that this finding was probably influenced by extreme outlier values³⁶. In our much larger cohort we found no relation with bronchial responsiveness or lung function measured later in childhood. Whilst we carried out multiple statistical comparisons, we were testing an *a priori* hypothesis with respect to specific antioxidant genes, and although we explored possible interactions with various phenotypic outcomes, our primary outcomes of interest were asthma and wheezing, as 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 of equal magnitude in those children who were not included, which seems unlikely, especially as our findings have been replicated in other cohorts. Whilst 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 sensitised to 26 other allergens in this cohort³⁷, 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, using a propensity score approach. Confounding by indication is of potential concern, as 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 the latter 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 migraine³⁸, 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 behavioural factors linked to acetaminophen use³⁹. With respect to the main findings of this study, namely the interactions between maternal genotype and prenatal acetaminophen exposure,

whilst 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. In order to confirm this, experimental studies in animal models could provide supportive evidence, although definitive evidence in humans can only come from randomised controlled trials⁴⁰, which will be challenging to carry out.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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.

The UK Medical Research Council, the Wellcome Trust and the University of Bristol provide core support for ALSPAC. This study was funded by the British Lung Foundation. SOS was an Asthma UK Senior Research Fellow.

Abbreviations

Nrf2	Nuclear erythroid 2 p45-related factor 2
GST	Glutathione-S-transferase
ALSPAC	Avon Longitudinal Study of Parents and Children (ALSPAC)

References

- (1). Shaheen SO, Sterne JA, Songhurst CE, Burney PG. Frequent paracetamol use and asthma in adults. *Thorax*. 2000; 55:266–270. [PubMed: 10722764]
- (2). Newson RB, Shaheen SO, Chinn S, Burney PGJ. Paracetamol sales and atopic disease in children and adults: an ecological analysis. *Eur Respir J*. 2000; 16:817–823. [PubMed: 11153577]
- (3). Etminan M, Sadatsafavi M, Jafari S, Doyle-Waters M, Aminzadeh K, FitzGerald JM. Acetaminophen Use and the Risk of Asthma in Children and Adults. *Chest*. 2009; 136:1316–1323. [PubMed: 19696122]
- (4). Beasley R, Clayton T, Crane J, von Mutius E, Lai CK, Montefort S, et al. Association between paracetamol use in infancy and childhood, and risk of asthma, rhinoconjunctivitis, and eczema in children aged 6–7 years: analysis from Phase Three of the ISAAC programme. *The Lancet*. 2008; 372:1039–1048.
- (5). Perzanowski M, Miller R, Tang D, Ali D, Garfinkel R, Chew G, et al. Prenatal acetaminophen exposure and risk of wheeze at age 5 years in an urban, low-income cohort. *Thorax*. 2010; 65:118–123. [PubMed: 19850963]
- (6). Shaheen SO, Newson RB, Sherriff A, Henderson AJ, Heron JE, Burney PGJ, et al. Paracetamol use in pregnancy and wheezing in early childhood. *Thorax*. 2002; 57:958–963. [PubMed: 12403878]

- (7). Shaheen SO, Newson RB, Henderson AJ, Headley JE, Stratton FD, Jones RW, et al. Prenatal paracetamol exposure and risk of asthma and elevated immunoglobulin E in childhood. *Clin Exp Allergy*. 2005; 35:18–25. [PubMed: 15649261]
- (8). Rebordosa C, Kogevinas M, Sorensen HT, Olsen J. Prenatal exposure to paracetamol and risk of wheezing and asthma in children: A birth cohort study. *Int J Epidemiol*. 2008; 37:583–590. [PubMed: 18400839]
- (9). Persky V, Piorkowski J, Hernandez E, Chavez N, Wagner-Cassanova C, Vergara C, et al. Prenatal exposure to acetaminophen and respiratory symptoms in the first year of life. *Annals of Allergy, Asthma, & Immunology*. 2008; 101:271–278.
- (10). Cho HY, Reddy SP, Kleeberger SR. Nrf2 Defends the Lung from Oxidative Stress. *Antioxidants & Redox Signaling*. 2006; 8:76–87. [PubMed: 16487040]
- (11). Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, et al. High Sensitivity of Nrf2 Knockout Mice to Acetaminophen Hepatotoxicity Associated with Decreased Expression of ARE-Regulated Drug Metabolizing Enzymes and Antioxidant Genes. *Toxicol Sci*. 2001; 59:169–177. [PubMed: 11134556]
- (12). Chan K, Han XD, Kan YW. An important function of Nrf2 in combating oxidative stress: Detoxification of acetaminophen. *PNAS*. 2001; 98:4611–4616. [PubMed: 11287661]
- (13). Reisman SA, Csanaky IL, Aleksunes LM, Klaassen CD. Altered Disposition of Acetaminophen in Nrf2-null and Keap1-knockdown Mice. *Toxicol Sci*. 2009; 109:31–40. [PubMed: 19246624]
- (14). Henderson CJ, Wolf CR, Kitteringham N, Powell H, Otto D, Park BK. Increased resistance to acetaminophen hepatotoxicity in mice lacking glutathione S-transferase Pi. *Proc Natl Acad Sci U S A*. 2000; 97:12741–12745. [PubMed: 11058152]
- (15). Golding J, Pembrey M, Jones R. ALSPAC Study Team. ALSPAC - The Avon Longitudinal Study of Parents and Children. I Study Methodology *Paediatric & Perinatal Epidemiology*. 2001; 15:74–87. [PubMed: 11237119]
- (16). Chinn S, Rona RJ. Height and age adjustment for cross sectional studies of lung function in children aged 6–11 years. *Thorax*. 1992; 47:707–714. [PubMed: 1440464]
- (17). Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax*. 1983; 38:760–765. [PubMed: 6648855]
- (18). Lu B, Zanutto E, Hornik R, Rosenbaum PR. Matching with doses in an observational study of a media campaign against drug abuse. *Journal of the American Statistical Association*. 2001; 96:1245–1253. [PubMed: 25525284]
- (19). Yamamoto T, Yoh K, Kobayashi A, Ishii Y, Kure S, Koyama A, et al. Identification of polymorphisms in the promoter region of the human NRF2 gene. *Biochem Biophys Res Commun*. 2004; 321:72–79. [PubMed: 15358217]
- (20). Marzec JM, Christie JD, Reddy SP, Jedlicka AE, Vuong H, Lanken PN, et al. Functional polymorphisms in the transcription factor NRF2 in humans increase the risk of acute lung injury. *FASEB J*. 2007; 21:2237–2246. [PubMed: 17384144]
- (21). Rose-Zerilli MJ, Barton SJ, Henderson AJ, Shaheen SO, Holloway JW. Copy-Number Variation Genotyping of GSTT1 and GSTM1 Gene Deletions by Real-Time PCR. *Clin Chem*. 2009; 55:1680–1685. [PubMed: 19589847]
- (22). Stata Statistical Software. College Station. TX: StataCorp LP; 2009.
- (23). Rebordosa C, Kogevinas M, Bech BH, Sorensen HT, Olsen J. Use of acetaminophen during pregnancy and risk of adverse pregnancy outcomes. *Int J Epidemiol*. 2009; 38:706–714. [PubMed: 19332503]
- (24). Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *American Journal of Epidemiology*. 2002; 155:176–184. [PubMed: 11790682]
- (25). Cho HY, Kleeberger SR. Nrf2 protects against airway disorders. *Toxicol Appl Pharmacol*. 2010; 244:43–56. [PubMed: 19646463]
- (26). Rangasamy T, Guo J, Mitzner WA, Roman J, Singh A, Fryer AD, et al. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. *J Exp Med*. 2005; 202:47–59. [PubMed: 15998787]

- (27). Siedlinski M, Postma D, Boer J, van der Steege G, Schouten J, Smit H, et al. Level and course of FEV1 in relation to polymorphisms in NFE2L2 and KEAP1 in the general population. *Respiratory Research*. 2009; 10:73. [PubMed: 19671143]
- (28). Coles B, Wilson I, Wardman P, Hinson JA, Nelson SD, Ketterer B. The spontaneous and enzymatic reaction of N-acetyl-p-benzoquinonimine with glutathione: A stopped-flow kinetic study. *Arch Biochem Biophys*. 1988; 264:253–260. [PubMed: 3395122]
- (29). Wolf CR, Park BK, Kitteringham N, Otto D, Henderson CJ. Functional and genetic analysis of glutathione S-transferase δ . *Chemico-Biological Interactions*. 2001; 133:280–284.
- (30). Levy G, Garrettson LK, Soda DM. Letter: Evidence of placental transfer of acetaminophen. *Pediatrics*. 1975; 55:895. [PubMed: 1134892]
- (31). Rollins DE, von Bahr C, Glaumann H, Moldeus P, Rane A. Acetaminophen: potentially toxic metabolite formed by human fetal and adult liver microsomes and isolated fetal liver cells. *Science*. 1979; 205:1414–1416. [PubMed: 38505]
- (32). Larrey D, Letteron P, Foliot A, Descatoire V, Degott C, Geneve J, et al. Effects of pregnancy on the toxicity and metabolism of acetaminophen in mice. *J Pharmacol Exp Ther*. 1986; 237:283–291. [PubMed: 3083096]
- (33). Dimova S, Hoet PH, Dinsdale D, Nemery B. Acetaminophen decreases intracellular glutathione levels and modulates cytokine production in human alveolar macrophages and type II pneumocytes in vitro. *International Journal of Biochemistry & Cell Biology*. 2005; 37:1727–1737. [PubMed: 15878691]
- (34). Nuttall SL, Khan JN, Thorpe GH, Langford N, Kendall MJ. The impact of therapeutic doses of paracetamol on serum total antioxidant capacity. *Journal of Clinical Pharmacy & Therapeutics*. 2003; 28:289–294. [PubMed: 12911681]
- (35). Davey G, Berhane Y, Duncan P, Aref-Adib Golnar, Britton J, Venn A. Use of acetaminophen and the risk of self-reported allergic symptoms and skin sensitization in Butajira, Ethiopia. *J Allergy Clin Immunol*. 2005; 116:863–868. [PubMed: 16210062]
- (36). Bisgaard H, Loland L, Holst KK, Pipper CB. Prenatal determinants of neonatal lung function in high-risk newborns. *J Allergy Clin Immunol*. 2009; 123:651–657. [PubMed: 19152964]
- (37). Roberts G, Peckitt C, Northstone K, Strachan D, Lack G, Henderson J, et al. Relationship between aeroallergen and food allergen sensitization in childhood. *Clin Exp Allergy*. 2005; 35:933–40. [PubMed: 16008681]
- (38). Chen TC, Leviton A. Asthma and eczema in children born to women with migraine. *Arch Neurol*. 1990; 47:1227–1230. [PubMed: 2241619]
- (39). Shaheen SO, Newson RB, Davey-Smith G, Henderson AJ. Prenatal paracetamol exposure and asthma: further evidence against confounding. *Int J Epidemiol*. 2010; 39:790–794.
- (40). Barr RG. Does paracetamol cause asthma in children? Time to remove the guesswork. *The Lancet*. 2008; 372:1011–1012.

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 usage and asthma in western populations.

Capsule summary

This study reports that the relation between maternal use of acetaminophen in pregnancy and childhood asthma may be modified by maternal antioxidant gene polymorphisms, strengthening evidence that this association may be causal.

Table 1
Adjusted effects of maternal acetaminophen use in early and late pregnancy on asthma and related phenotypes

Outcome	N	Early pregnancy			N	Late pregnancy		
		Adj. Estimate*	(95% CI)	P		Adj. Estimate*	(95% CI)	P
Odds ratios:								
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 ratios:								
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 differences:								
Adjusted FEV ₁ (SDs)	6436	-0.01	(-0.06, 0.04)	.82	6218	0.00	(-0.05, 0.05)	.87
Adjusted FVC (SDs)	6536	-0.01	(-0.06, 0.04)	.64	6314	-0.02	(-0.07, 0.03)	.55
Adjusted FEF ₂₅₋₇₅ (SDs)	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, body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7, infant acetaminophen use).

Table 2
Associations between maternal use of acetaminophen in early and late pregnancy and children's asthma stratified by maternal genotype

	Maternal acetaminophen use							
	Early pregnancy				Late pregnancy			
	N	Odds ratio*	(95% CI)	P	N	Odds ratio*	(95% CI)	P
Nrf2								
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
GSTT1 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
GSTM1 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
GSTP1								
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, body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7, infant acetaminophen use).

Table 3
Associations between maternal use of acetaminophen in early and late pregnancy and children's wheezing stratified by maternal genotype

		Maternal acetaminophen use							
		Early pregnancy				Late pregnancy			
	N	Odds ratio*	(95% CI)	P	N	Odds ratio*	(95% CI)	P	
Nrf2									
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	
GSTT1 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	
GSTM1 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	
GSTP1									
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, body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, BMI at age 7, infant acetaminophen use).

Table 4
Unadjusted and adjusted effects of infant acetaminophen use on childhood asthma and related phenotypes

Odds ratios:							
<i>Outcome</i>	<i>N</i>	<i>Unadj.</i>	<i>(95% CI)</i>	<i>P</i>	<i>Adj.*</i>	<i>(95% CI)</i>	<i>P</i>
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 ratios:							
<i>Outcome</i>	<i>N</i>	<i>Unadj.</i>	<i>(95% CI)</i>	<i>P</i>	<i>Adj.*</i>	<i>(95% CI)</i>	<i>P</i>
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 differences:							
<i>Outcome</i>	<i>N</i>	<i>Unadj.</i>	<i>(95% CI)</i>	<i>P</i>	<i>Adj.*</i>	<i>(95% CI)</i>	<i>P</i>
Adjusted FVC (SDs)	6327	-0.01	(-0.04, 0.03)	.7	-0.01	(-0.04, 0.03)	.69
Adjusted FEV ₁ (SDs)	6231	0.00	(-0.03, 0.04)	.8	0.01	(-0.03, 0.04)	.66
Adjusted FEF ₂₅₋₇₅ (SDs)	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, body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7).

Table 5
Adjusted effects of infant acetaminophen use on childhood asthma and related phenotypes according to wheezing in the first 6 months

Odds ratios:	No wheeze				Wheeze			
	N	OR*	(95% CI)	P	N	OR*	(95% CI)	P
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, body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7).

Table 6
Associations between infant use of acetaminophen and childhood asthma stratified by child genotype

	N	OR*	(95% CI)	P
GSTT1 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
GSTM1 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
GSTP1				
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
Nrf2				
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, body mass index, ethnicity, age, parity, history of asthma, eczema, rhinoconjunctivitis, migraine); sex of child, season of birth, multiple pregnancy, gestational age, birthweight, head circumference, birth length; postnatal factors (breast feeding, day care, pets, damp/mould, environmental tobacco smoke exposure, number of younger siblings, infant antibiotic use, BMI at age 7).