***TRPA1* gene polymorphisms and childhood asthma**

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Abstract

Background: Animal data have suggested that the transient receptor potential ankyrin-1 (TRPA1) ion channel, an oxidant sensor, plays a key role in promoting airway inflammation in asthma and may mediate effects of acetaminophen on asthma, yet confirmatory human data are lacking.

Objective: To study associations of *TRPA1* gene variants with childhood asthma and total IgE, and interactions between *TRPA1* and prenatal acetaminophen exposure on these outcomes.

Methods: We analysed associations between 31 *TRPA1* single nucleotide polymorphisms (SNPs) and current doctor-diagnosed asthma and total IgE at 7.5 years in the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort. We sought to confirm the most significant associations with comparable outcomes in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) and Generation R birth cohorts. In ALSPAC we explored interactions with prenatal acetaminophen exposure.

Results: In ALSPAC there was strong evidence for association between six SNPs and asthma: rs959974 and rs1384001 (per allele odds ratio for both: 1.30 (95% CI: 1.15-1.47), P=0.00001), rs7010969 (OR 1.28 (1.13-1.46), P=0.00004), rs3735945 (OR 1.30 (1.09-1.55), P=0.003), rs920829 (OR 1.30 (1.09-1.54), P=0.004) and rs4738202 (OR 1.22 (1.07-1.39), P=0.004). In a meta-analysis across the three cohorts the pooled effect estimates confirmed that all six SNPs were positively associated with asthma. In ALSPAC, *TRPA1* associations with asthma were not modified by prenatal acetaminophen exposure, although associations with IgE were.

Conclusion: This study suggests that TRPA1 may play a role in the development of childhood asthma. Activation of TRPA1 is unlikely to explain the association between prenatal acetaminophen exposure and asthma. (254 words)

Clinical implications

In terms of therapeutic implications, these data lend further support to the proposition that TRPA1 antagonists may have promising potential in asthma.

Capsule summary

This epidemiological study suggests, for the first time, that TRPA1 may play a role in the development of childhood asthma.

Key words: TRPA1, asthma, ALSPAC, PIAMA, Generation R, acetaminophen, prenatal exposure, birth cohort, genotype, gene-environment interaction

Abbreviations used:

TRPA1: Transient receptor potential ankyrin-1

ALSPAC: Avon Longitudinal Study of Parents and Children

NAPQI: N-acetyl-p-benzo-quinoneimine

PIAMA: Prevention and Incidence of Asthma and Mite Allergy

SNP: Single nucleotide polymorphism

PAF: Population-attributable fraction

LD: Linkage disequilibrium

Introduction

The transient receptor potential ankyrin-1 (TRPA1) ion channel is expressed on peripheral endings of primary afferent neurons and is a highly conserved sensor of noxious reactive electrophiles; these form covalent adducts with the receptor to activate the neurons[1]. In particular, TRPA1 is a major oxidant sensor in the airways[2], sensing exogenous airborne irritants as well as endogenous by-products of oxidative stress[3]. In keeping with this function, the TRPA1 receptor is thought to play a key role in the cough reflex[4] and in promoting airway inflammation in asthma[3;5]. Experiments using knock-out mice and TRPA1 antagonists have shown that TRPA1 plays a critical role in allergic and non-allergic neurogenic airway inflammation and hyperreactivity[6;7].

Following our initial discovery of an association between frequent acetaminophen (paracetamol) use and asthma in adults[8], we reported that maternal use of acetaminophen in pregnancy was associated with an increased risk of childhood asthma, wheezing and elevated total IgE in the Avon Longitudinal Study of Parents and Children (ALSPAC)[9], and that the relation between prenatal acetaminophen exposure and asthma was modified by maternal antioxidant gene polymorphisms[10]. Other birth cohorts have confirmed a link between prenatal acetaminophen exposure and wheezing[11]. Nassini *et al* subsequently showed in a rodent model that systemic administration of therapeutic doses of acetaminophen led to generation of its electrophilic and reactive metabolite, *N*-acetyl-*p*-benzo-quinoneimine (NAPQI), in the lung which, in turn, caused neurogenic airway inflammation through activation of TRPA1; they proposed that this mechanism might explain the epidemiological link between frequent acetaminophen use and asthma in humans[12].

Despite the plausibility of TRPA1 playing a crucial role in asthma, and supportive evidence in animals, confirmatory data in humans have been lacking. In ALSPAC we therefore investigated whether *TRPA1* (8q13) gene variants are associated with childhood asthma and IgE, and sought to obtain confirmatory evidence for the most significant SNP associations in the Prevention and Incidence of Asthma and Mite allergy (PIAMA) and Generation R birth cohorts. In ALSPAC we also explored whether *TRPA1* associations with asthma and IgE were modified by prenatal exposure to acetaminophen.

Methods

ALSPAC (UK; discovery cohort)

Subjects

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort that recruited 14,541 predominantly white pregnant women resident in Avon, UK with expected dates of delivery 1st April 1991 to 31st December 1992. Of these pregnancies there were 14,676 fetuses, resulting in 14,062 live births and 13,988 children who were alive at one year of age. 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[13;14] and further information can be found at: http://www.alspac.bris.ac.uk. The study website contains details of all the data that are available through a fully searchable data dictionary: http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/. Ethics approval for all aspects of data collection was obtained from the ALSPAC Ethics and Law Committee (IRB 00003312) and the Local Research Ethics Committees.

Outcomes

The primary outcome of interest was current doctor-diagnosed asthma at 7.5 years. 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?'. Children were defined as cases 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.

Serum total IgE (kU/l) was measured by fluoroimmunoassay using the Pharmacia UNICAP system (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) at 7 years.

Information on prenatal acetaminophen exposure

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. We used this information to define use of acetaminophen (Yes/No) in early (<18-20 weeks) and late (20-32 weeks) pregnancy.

Genotyping and selection of *TRPA1* SNPs

DNA samples were extracted from lymphoblastoid cell lines, cord blood, or venous blood collected at 7 years of age, with a small number extracted from venous blood collected at 43-61 months. A total of 9,912 subjects were genotyped at 500,527 SNPs using the Illumina HumanHap550 quad genome-wide SNP genotyping platform. In order to obtain the most robust and highest quality GWAS data, we applied various rigorous exclusion criteria (eg excluding those with evidence of cryptic relatedness and those of non-European ancestry); after these measures genotype data were available for 8,365 unrelated individuals (see Online Supplement for further details of all exclusion criteria).

We identified 29 SNPs in *TRPA1* (8q13) which had been included in a genetic association study of cough[15]. The participating cohorts in that study were part of a large European genome-wide association study (GWAS) of asthma (the GABRIEL consortium)[16], and all SNPs within the gene region had been selected; this allowed the capture of the majority of common (frequency ≥5%) haplotype variations of the

gene[15 16]. In addition we identified 11 SNPs (four of which had already been selected) associated with various pain phenotypes[17-19] and with menthol preference in smokers[20]. Of the 36 potential SNPs, five had not been typed or could not be imputed, leaving 31 SNPs to be analysed. Of these SNPs, 21 were genotyped and 10 were imputed (see Online Table E1 for details). Where genotyped data were missing these were replaced by imputed data if possible (see Online Supplement for further details of imputation methods and quality control measures for imputed genotypes).

Statistical analyses of ALSPAC data

Although the ALSPAC population is largely white, and the GWAS dataset only included individuals of European ancestry, in order to further reduce the possibility of confounding by population substructure we excluded mother-child pairs from all analyses if the mother's reported ethnicity was non-white or unknown (14.1% of the cohort). We used logistic regression to analyse relations of child *TRPA1* genotype with asthma, and linear regression to analyse associations with log-transformed total IgE. All analyses were carried out using Stata (version 10.1). Univariate gene main effects were evaluated as continuous per allele effects and using between genotype comparisons (homozygotes for effect allele versus homozygotes for reference allele, and heterozygotes versus homozygotes for reference allele). We used Haploview[21] to compute linkage disequilibrium (LD) statistics for the 31 *TRPA1* SNPs of interest. The population-attributable fraction (PAF) was calculated in Stata using the formula: PAF=1-PUF, where the PUF is the population unattributable fraction (the fraction of asthma that would remain in the population if everybody was homozygous for the reference (non-effect) allele[22]). We used the Nyholt approach[23](http://neurogenetics.qimrberghofer.edu.au/SNPSpD/) updated by Li and

Ji[24] to estimate the effective number of independent marker loci in our data (12.8 independent marker loci out of 31) and the threshold required to keep type I error rate at 5% after adjusting for multiple testing (P value=0.05/12.8=0.004).

PIAMA and Generation R (Netherlands)

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort is a multi-centre study that selected 4146 pregnant women (1327 with and 2819 without allergy) in The Netherlands in 1996 and 1997. The study protocol has been described previously[25;26]. The Generation R Study is a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, The Netherlands[27;28]. All children were born between April 2002 and January 2006, and currently followed until young adulthood. Of all eligible children in the study area, 61% (n = 9,901) were participating in the study at birth. Current doctor-diagnosed asthma at 8 years and at 6 years was defined in PIAMA and Generation R, respectively (see Online Supplement for further details of questionnaire-based information, genotyping and imputation methods, and ethics).

The smaller size (and hence reduced statistical power) of the PIAMA and Generation R cohorts compared with ALSPAC limited our ability to replicate the ALSPAC 'top hits' for asthma in each of the other two cohorts individually. We therefore meta-analysed the associations between the SNPs which were most significantly associated with asthma in ALSPAC across the three cohorts. We used a fixed effects model using the method of Mantel and Haenszel (weights are calculated using the inverse variance method).

Other European asthma studies

In other European studies which had been included in the GABRIEL asthma GWAS study[16] we explored associations between doctor-diagnosed asthma ‘ever’ (of childhood onset) and the *TRPA1* SNPs most significantly associated with asthma in ALSPAC. We carried out these subsidiary analyses using publicly available data from GABRIEL, and meta-analysed the data using a fixed effects model, distinguishing the few population-based studies which were exclusively of children from all other studies; the latter included a heterogeneous mixture of family studies, clinic-based studies, and adult studies which had relied on recall to determine asthma of childhood onset.

Results

In ALSPAC, information on current doctor-diagnosed asthma at age 7.5 years was obtained for 7,221 children. After excluding mother-child pairs if maternal ethnicity was non-white or unknown, and applying quality criteria to imputed genotype data, *TRPA1* genotype data were available for 6,901 children, generating a final sample of 5,141 white children with complete data on asthma and genotype. Data on total IgE and genotype were available for 3,834 children.

In the sample of 5,141 children, 614 (11.9%) children had current doctor-diagnosed asthma at age 7.5 years. 53.9% and 42.3% of children were exposed to acetaminophen *in utero* during early and late pregnancy, respectively. *TRPA1* genotype data are summarised in Table E1. We found no evidence that *TRPA1* genotype frequencies deviated from Hardy-Weinberg equilibrium for the 31 SNPs of interest ($P>0.05$).

In PIAMA, information on current doctor-diagnosed asthma at age 8 years was obtained for 3,253 children, and TRPA1 genotype data were available for 1,968 children, generating a final sample of 1,877 white children with data on asthma and genotype, of whom 89 (4.7%) had current doctor-diagnosed asthma at age 8 years. In Generation R, data on TRPA1 genotype and current doctor-diagnosed asthma at age 6 years were available for 2,073 children, after excluding twins and restricting to Caucasians only, based on genetic ancestry. Of these, 64 children (3.1%) had current doctor-diagnosed asthma.

<u>Gene main effects in ALSPAC</u>

Table 1 shows the per allele associations between *TRPA1* genotypes and asthma in ALSPAC. Of the 31 SNPs tested, 13 were associated with asthma ($P<0.05$). The six SNPs (five genotyped, one imputed) that were most significantly associated with

asthma (P<0.005) were: rs959974 and rs1384001 (per allele odds ratio for both SNPs: 1.30 (95% CI: 1.15-1.47), P=0.00001), rs7010969 (OR 1.28 (1.13-1.46), P=0.00004), rs3735945 (OR 1.30 (1.09-1.55), P=0.003), rs920829 (OR 1.30 (1.09-1.54), P=0.004) and rs4738202 (OR 1.22 (1.07-1.39), P=0.004). Adjustment for multiple testing (taking into account which of the 31 tests were independent, given SNPs in LD with each other) suggested that associations with these six SNPs (and especially the first four) were unlikely to have arisen by chance (adjusted P value threshold 0.004). Additional effect estimates using between genotype comparisons for these six SNPs in relation to asthma are shown in Table 2. This shows that, for four of these SNPs, children who were homozygous for the effect allele were approximately 70% more likely to have asthma than children who were homozygous for the reference allele. For the other two SNPs the number of children who were homozygous for the effect allele was very small and confidence limits around the effect estimates were very wide. Of the 31 SNPs tested, only three (rs959974, rs1384001, rs4738202) were nominally associated with total IgE (P<0.05) (Table E2).

Figure E1 in the online supplement shows LD (r^2) between the 31 *TRPA1* SNPs, 29 of which are located in four LD blocks. Of the six SNPs most significantly associated with asthma, two (rs959974 and rs1384001) were in one block, rs4738202 was in another block, and rs7010969, rs3735945 and rs920829 were in a third block. When we included one SNP from each of the three blocks (rs959974, rs4738202, rs920829) in the regression model for asthma, the mutually adjusted per-allele effect estimates were all substantially attenuated, suggesting collinearity (data not shown). We chose three of the most significantly associated SNPs (rs959974, rs7010969 and rs4738202) to separately estimate the proportion of asthma in the population attributable to

297 *TRPA1* genotype (PAF). The PAF estimates were, respectively, 21.7% (95% CI: 9.6-
298 32.2; P=0.001), 29.1% (12.5-42.6; P=0.001) and 30.7% (7.7-47.9; P=0.012).
299
300 Gene main effects in PIAMA and Generation R and meta-analysis
301 Table 2 also shows the associations between the six SNPs, which were most
302 significantly associated with asthma in ALSPAC, and asthma in the PIAMA and
303 Generation R cohorts. In PIAMA there was some evidence for association with
304 asthma for three of these SNPs ($P \leq 0.05$), with effect estimates that were larger than
305 those in ALSPAC. In Generation R none of the six SNPs were associated with
306 asthma. Figure 1 shows the Forest plots for the weighted per-allele associations of the
307 six SNPs with asthma when we carried out a meta-analysis across the three birth
308 cohorts. For all six SNPs the pooled effect estimates confirmed positive associations
309 with asthma.
310
311 Gene main effects in other European asthma studies
312 Figures E2-E6 online show Forest plots for the meta-analysis of the associations
313 between *TRPA1* and childhood-onset asthma across GABRIEL studies, for five of the
314 six SNPs that were most significantly associated with asthma in ALSPAC (rs920829
315 was not genotyped in GABRIEL; it was imputed in ALSPAC, but is in strong LD
316 with rs3735945, as evidenced by the identical effect estimates for these two SNPs in
317 ALSPAC, PIAMA and Generation R (Table 2)). The plots compare associations with
318 current doctor-diagnosed asthma in ALSPAC and PIAMA versus associations with
319 doctor-diagnosed asthma 'ever' (of childhood-onset) across other GABRIEL studies,
320 with three studies which were exclusively of children separated from remaining
321 studies. The pooled effect estimates do not confirm associations with asthma 'ever'.

Furthermore, there was evidence of substantial heterogeneity in the effect estimates for the three childhood GABRIEL studies.

Acetaminophen analyses in ALSPAC

For the 13 SNPs associated with asthma ($P<0.05$) we stratified the per allele associations by early and late gestation acetaminophen exposure. Associations were similar in exposed and unexposed children for the six SNPs which were most significantly associated with asthma overall (Table 3) and for the remaining 7 SNPs (data not shown). For the three SNPs associated with IgE ($P<0.05$) we similarly stratified the per allele associations by prenatal acetaminophen exposure (Table 4). *TRPA1* was associated with IgE amongst children who were exposed, especially in later gestation, but was not associated with IgE amongst non-exposed children (P interaction 0.02 for rs959974 and rs1384001, and 0.06 for rs4738202).

Discussion

We found strong evidence for an association between *TRPA1* polymorphisms and asthma in children at 7-8 years of age in the population-based ALSPAC birth cohort. Furthermore, the association between *TRPA1* and IgE, but not asthma, was stronger in children exposed prenatally to acetaminophen than in non-exposed children. Of the six SNPs most significantly associated with asthma in ALSPAC, three showed some evidence of association (and larger effect estimates) with a similar asthma phenotype in the PIAMA birth cohort, whilst none of the six SNPs were associated with asthma at 6 years in Generation R. However, both PIAMA and Generation R were considerably smaller, and had a lower prevalence of current asthma, than ALSPAC, and hence they lacked statistical power to replicate findings individually. When we meta-analysed across all three birth cohorts the pooled effect estimates confirmed associations with asthma overall. Given the *a priori* selection of SNPs, the level of statistical significance for the six 'top hits' in the ALSPAC discovery dataset (even after adjustment for multiple testing), and supportive evidence in PIAMA and following meta-analysis across all three cohorts, we believe these results may represent a causal influence of the *TRPA1* gene on the risk of active childhood asthma. Furthermore, other genes in the vicinity of *TRPA1* are unlikely to explain our findings as there is little apparent LD extending between *TRPA1* and other nearby genes (1000 Genomes Phase 1 CEU (www.1000genomes.org)). To our knowledge these findings are novel, and suggest that TRPA1 may play a role in the pathogenesis of asthma in humans, and specifically in the development of childhood asthma. A previous study has linked a *TRPV1* gene variant to childhood wheezing[29], but associations between *TRPA1* polymorphisms and childhood asthma prevalence have not been reported; whilst a recent study reported correlations between two *TRPA1*

polymorphisms and asthma control in children with asthma[30], it was underpowered and statistical evidence was weak.

Importance of asthma phenotype

There is likely to be genetic heterogeneity of asthma phenotypes in childhood[31], as has been demonstrated for adult asthma phenotypes[32]. This may partly explain why *TRPA1* was not associated with asthma in the other European studies. A limitation of the GABRIEL asthma GWAS was that the asthma 'ever' phenotype was not directly comparable to the 'current' asthma phenotype used in ALSPAC, PIAMA and Generation R; a doctor diagnosis of asthma 'ever' is likely to comprise many different phenotypes or endotypes which, when analysed together, may lead to dilution of effects of genetic variants[33]. For example, in children, 'asthma ever' may capture early transient childhood wheezing which did not persist. We confirmed that the effect estimates for the association between *TRPA1* and asthma were smaller in ALSPAC, and especially in PIAMA, when we analysed 'ever' asthma rather than 'current' asthma in these cohorts. Other possible reasons for the lack of association across the other European studies include differences in how cases were selected in the various GABRIEL studies (eg clinic versus general population samples) which may have contributed to heterogeneity of the asthma phenotype; unreliability of recall of childhood onset asthma amongst the adult studies in GABRIEL; and variation in the prevalence of environmental exposures that interact with the gene across different European populations[34].

Mechanisms

Given that reactive oxygen species are thought to play an important role in the pathogenesis of airways disease[35], and the TRPA1 receptor is an important oxidant sensor expressed on sensory neurons innervating the airways[2], it seems very plausible that TRPA1 may play a critical role in asthma pathogenesis. Strong supportive evidence for this comes from animal experiments. Activation of TRPA1 can, through release of neuropeptides including substance P and calcitonin gene-related peptide, promote neurogenic airway inflammation[3;5]. Conversely, in murine models of airway inflammation induced by asthma risk factors, including allergen, cigarette smoke and acetaminophen, deletion or antagonism of TRPA1 has been shown to reduce airway inflammation and hyper-reactivity[6;12;36]. However, as neurogenic inflammation has not been demonstrated in human asthma, there are two other mechanisms to consider. First, TRPA1 may also influence airway inflammation non-neuronally; such a pathway has been confirmed in animals[37], and recent *in vitro* studies have shown that *TRPA1* is functionally expressed in human lung, including pulmonary epithelial cells[37;38], smooth muscle cells[37], and lung fibroblasts[38]. Second, a neuronal reflex mechanism may be involved. Experiments in rodents suggest that TRPA1 is involved in the late asthmatic response, and it has been proposed that allergen challenge can trigger airway sensory nerves via the activation of TRPA1 channels, which in turn initiates a central reflex event leading to a parasympathetic cholinergic constrictor response[39].

In order to determine whether TRPA1 might be on the causal path between maternal use of acetaminophen in pregnancy and increased risk of childhood asthma and elevated total IgE, we explored whether the association between *TRPA1* genotype and

asthma was stronger in those exposed prenatally to acetaminophen. The lack of effect modification suggests that, even if fetal TRPA1 is activated by exposure to the metabolite of acetaminophen (NAPQI)[12] *in utero*, this mechanism is unlikely to explain the association between prenatal acetaminophen and asthma. In contrast, we did find some evidence that the association between *TRPA1* genotype and IgE was stronger in children who were prenatally exposed to acetaminophen. Whilst this apparent interaction is intriguing, it may be a chance finding and we cannot offer a mechanistic explanation. We speculate that other prenatal and postnatal oxidant exposures may be more important than acetaminophen as activators of TRPA1, thus contributing to the association we have found between *TRPA1* genotype and childhood asthma.

Conclusions and future work

Our findings suggest, for the first time, that TRPA1 may play an important role in the development of childhood asthma. Furthermore, the proportion of childhood asthma in the population attributable to *TRPA1* (PAF), assuming a causal relation, may be substantial. We speculate that this might reflect the fact that TRPA1 is activated by an array of agonists. In terms of therapeutic implications, these data lend further support to the proposition that TRPA1 antagonists may have promising potential in asthma[4]. By way of further epidemiological study, it is important that our findings are further replicated in adequately powered studies with comparable asthma phenotypes, and we plan to explore interactions between *TRPA1* and other oxidant exposures such as tobacco smoke and air pollution on childhood respiratory outcomes.

Contributors

SOS conceived the study analyses, searched the literature, supervised the ALSPAC analyses and drafted the manuscript. VG carried out the ALSPAC analyses, with additional contribution from RG; FND and GHK carried out the PIAMA analyses; HTD carried out the Generation R analyses; DPS carried out the meta-analysis of GABRIEL data. JWH and GHK advised on analysis and interpretation of genetic data; SMR was responsible for the ALSPAC genotyping; AJH was responsible for all respiratory and allergy phenotype data collection in ALSPAC; GHK and DSP were responsible for DNA, respiratory and allergy phenotype data collection in PIAMA and supervised data analyses; JCJ, VWVJ and LD were responsible for DNA, respiratory and allergy phenotype data collection in Generation R. All authors contributed to 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.

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476 **Table 1: Per-allele associations between child *TRPA1* SNPs and current doctor**
477 **diagnosed asthma at 7.5 years in ALSPAC**

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SNP	Position	Doctor diagnosed asthma at 7 years		
		N	OR (95% CI)	P value
rs12540984	72927920	5110	1.00 (0.84-1.18)	0.985
rs4738201	72930711	5140	1.16 (1.03-1.31)	0.013
rs6996723	72933632	5141	0.88 (0.75-1.04)	0.137
rs7827617	72934032	5141	1.21 (1.04-1.41)	0.013
rs959974	72935839	5141	1.30 (1.15-1.47)	0.00001
rs959976	72936145	5141	1.22 (1.05-1.42)	0.008
rs1384001	72936237	5141	1.30 (1.15-1.47)	0.00001
rs13279503	72939626	5116	1.08 (0.95-1.22)	0.222
rs4738202	72940861	5141	1.22 (1.07-1.39)	0.004
rs13280644	72948588	5141	0.82 (0.66-1.02)	0.075
rs13249568	72949209	5141	0.95 (0.83-1.09)	0.468
rs10504523	72951490	5141	0.95 (0.83-1.09)	0.484
rs1025926	72953158	5141	1.14 (1.00-1.30)	0.055
rs10504524	72955891	5141	0.95 (0.83-1.09)	0.479
rs13255063	72959535	5140	0.95 (0.83-1.09)	0.476
rs1025927	72963135	5138	0.82 (0.66-1.01)	0.067
rs1025928	72963258	5141	0.94 (0.83-1.07)	0.344
rs10504525	72965123	5141	1.06 (0.90-1.25)	0.494
rs3735942	72965973	5141	1.11 (0.98-1.26)	0.097
rs3735943	72966002	5141	0.88 (0.78-0.99)	0.040
rs10504526	72966552	5141	1.13 (1.01-1.28)	0.041
rs12548486	72971527	5138	1.11 (0.98-1.26)	0.102
rs10109581	72974329	5141	1.19 (1.05-1.36)	0.009
rs3735945	72974806	5141	1.30 (1.09-1.55)	0.003
rs920829	72977703	5136	1.30 (1.09-1.54)	0.004
rs1443952	72980652	5141	1.11 (0.98-1.25)	0.116
rs7010969	72982365	5141	1.28 (1.13-1.46)	0.00004
rs7011431	72982398	5141	1.20 (1.05-1.36)	0.008
rs4738206	72986348	5141	1.10 (0.97-1.25)	0.120
rs2278655	72987277	5038	1.01 (0.79-1.28)	0.964
rs13268757	72987638	5097	1.06 (0.89-1.25)	0.528

479

480 **Table 2: Associations between the six most significantly associated *TRPA1* SNPs in ALSPAC and current doctor diagnosed asthma at 7-8 years in ALSPAC and PIAMA, and**
481 **current doctor diagnosed asthma at 6 years in Generation R**

		ALSPAC			PIAMA			GENERATION R		
SNP	**Alleles**	**N**	**OR**	**p-value**	**N**	**OR**	**p-value**	**N**	**OR**	**p-value**
rs959974*	G/G	1,401	1.00		512	1.00		555	1.00	
	G/T	2,615	1.33 (1.07-1.65)	0.009	932	1.36 (0.77-2.38)	0.28	1,054	1.15 (0.61, 2.14)	0.67
	T/T	1,125	1.69 (1.32-2.16)	0.00001	433	1.82 (0.99-3.36)	0.053	464	1.39 (0.68, 2.82)	0.37
	Per allele		1.30 (1.15-1.47)	0.00001		1.35 (1.00-1.83)	0.052		1.18 (0.83, 1.68)	0.37
rs1384001†	C/C	1400	1.00		512	1.00		555	1.00	
	A/C	2,616	1.33 (1.07-1.65)	0.009	933	1.36 (0.78-2.38)	0.28	1,054	1.15 (0.61, 2.14)	0.67
	A/A	1125	1.69 (1.32-2.15)	0.00001	432	1.83 (0.99-3.37)	0.053	464	1.39 (0.68, 2.82)	0.37
	Per allele		1.30 (1.15-1.47)	0.00001		1.35 (1.00-1.83)	0.051		1.18 (0.83, 1.68)	0.37
rs4738202*	A/A	483	1.00		150	1.00		179	1.00	
	A/G	2,233	1.45 (1.02-2.05)	0.038	816	1.54 (0.54-4.41)	0.42	880	0.71 (0.30, 1.68)	0.44
	G/G	2,425	1.66 (1.18-2.34)	0.004	911	2.21 (0.79-6.20)	0.13	1,014	0.78 (0.34, 1.81)	0.57
	Per allele		1.22 (1.07-1.39)	0.004		1.45 (1.01-2.09)	0.042		0.96 (0.65, 1.41)	0.83
rs7010969†	A/A	827	1.00		299	1.00		324	1.00	
	A/C	2,477	1.43 (1.09-1.89)	0.010	920	1.09 (0.56-2.10)	0.80	1,005	1.08 (0.51, 2.32)	0.84
	C/C	1,837	1.74 (1.31-2.29)	0.00005	658	1.42 (0.73-2.77)	0.30	744	1.20 (0.55, 2.62)	0.65
	Per allele		1.28 (1.13-1.46	0.00004		1.23 (0.89-1.68)	0.21		1.10 (0.76, 1.59)	0.61
rs3735945†	C/C	4,067	1.00		1519	1.00		1,621	1.00	
	C/T	1,005	1.38 (1.13-1.68)	0.002	338	1.15 (0.67-1.95)	0.61	428	1.40 (0.80, 2.47)	0.24
	T/T	69	1.19 (0.59-2.41)	0.633	20	0.00 (0.00- -)¶	0.99	24	0.00 (0.00- -)¶	0.99
	Per allele		1.30 (1.09-1.55)	0.003		1.01 (0.61-1.65)	0.98		1.21 (0.71, 2.04)	0.48
rs920829#	C/C	4,066	1.00		1519	1.00		1,621	1.00	
	C/T	1001	1.37 (1.12-1.68)	0.002	338	1.15 (0.67-1.95)	0.61	428	1.40 (0.80, 2.47)	0.24
	T/T	69	1.19 (059-2.41)	0.634	20	0.00 (0.00- -)¶	0.99	24	0.00 (0.00- -)¶	0.99
	Per allele		1.30 (1.09-1.54)	0.004		1.01 (0.61-1.65)	0.98		1.21 (0.71, 2.04)	0.48

*Genotyped in ALSPAC and in PIAMA, and imputed in Generation R; †Genotyped in ALSPAC, and imputed in PIAMA and Generation R; #Imputed in ALSPAC and in PIAMA, and genotyped in Generation R; ¶No asthma cases in minor allele homozygote group in PIAMA and Generation R.

Table 3: Per-allele associations between the six most significantly associated *TRPA1* SNPs and current doctor diagnosed asthma, stratified by prenatal acetaminophen exposure during early and late gestation in ALSPAC

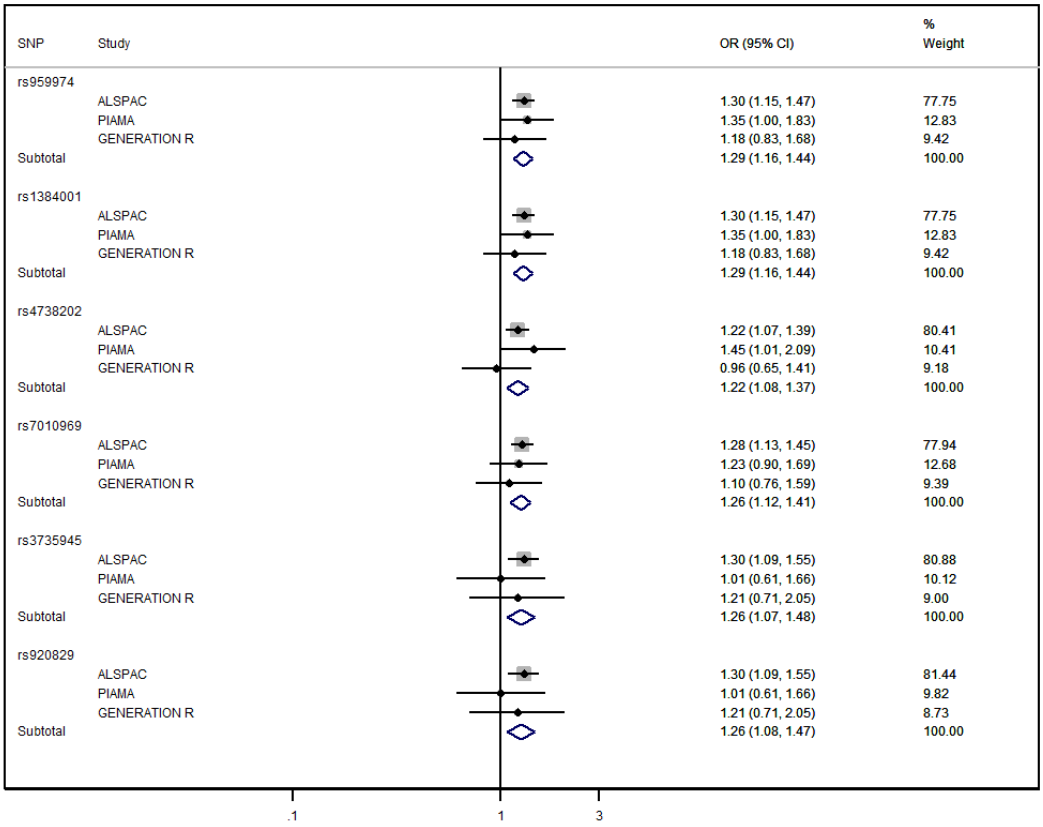
SNP	N	Acetaminophen early in pregnancy OR (95% C.I.)	p-value	N	Acetaminophen later in pregnancy OR (95% C.I.)	p-value
rs959974						
Exposed	2,734	1.29 (1.10-1.50)	0.002	2,118	1.26 (1.06-1.50)	0.008
Unexposed	2,338	1.37 (1.12-1.66)	0.002	2,889	1.31 (1.10-1.56)	0.002
		p-interaction	0.639		p-interaction	0.765
rs1384001						
Exposed	2,734	1.29 (1.10-1.50)	0.002	2,118	1.26 (1.06-1.50)	0.008
Unexposed	2,338	1.37 (1.12-1.66)	0.002	2,889	1.31 (1.10-1.56)	0.002
		p-interaction	0.643		p-interaction	0.760
rs4738202						
Exposed	2,734	1.22 (1.03-1.45)	0.024	2,118	1.23 (1.02-1.49)	0.031
Unexposed	2,338	1.24 (1.00-1.54)	0.049	2,889	1.18 (0.97-1.43)	0.090
		p-interaction	0.910		p-interaction	0.753
rs7010969						
Exposed	2,734	1.25 (1.07-1.47)	0.006	2,118	1.25 (1.05-1.50)	0.012
Unexposed	2,338	1.34 (1.09-1.64)	0.005	2,889	1.31 (1.09-1.57)	0.003
		p-interaction	0.621		p-interaction	0.738
rs3735945						
Exposed	2,734	1.15 (0.91-1.44)	0.242	2,118	1.31 (1.02-1.68)	0.036
Unexposed	2,338	1.59 (1.20-2.09)	0.001	2,889	1.24 (0.96-1.60)	0.096
		p-interaction	0.076		p-interaction	0.755
rs920829						
Exposed	2,732	1.15 (0.91-1.44)	0.238	2,114	1.30 (1.01-1.67)	0.041
Unexposed	2,335	1.57 (1.19-2.08)	0.001	2,888	1.24 (0.96-1.61)	0.093
		p-interaction	0.086		p-interaction	0.809

Table 4: Per-allele associations between the three most significantly associated *TRPA1* SNPs and total IgE, stratified by prenatal acetaminophen exposure during early and late gestation in ALSPAC

SNP	N	Acetaminophen early in pregnancy GMR* (95% C.I.)	p-value	N	Acetaminophen later in pregnancy GMR* (95% C.I.)	p-value
rs959974						
Exposed	2066	1.12 (1.01-1.24)	0.037	1587	1.22 (1.08-1.37)	0.001
Unexposed	1719	1.05 (0.94-1.17)	0.408	2149	1.01 (0.91-1.12)	0.849
		p-interaction	0.414		p-interaction	0.017
rs1384001						
Exposed	2066	1.12 (1.01-1.24)	0.037	1587	1.22 (1.08-1.37)	0.001
Unexposed	1719	1.05 (0.94-1.17)	0.402	2149	1.01 (0.91-1.12)	0.849
		p-interaction	0.418		p-interaction	0.016
rs4738202						
Exposed	2066	1.16 (1.03-1.29)	0.011	1587	1.21 (1.06-1.37)	0.003
Unexposed	1719	1.02 (0.91-1.15)	0.714	2149	1.03 (0.92-1.15)	0.585
		p-interaction	0.145		p-interaction	0.062

*Geometric Mean Ratio

Figure 1: Forest plots showing meta-analysis of the per-allele associations between the six *TRPA1* SNPs most significantly associated with asthma in ALSPAC and current asthma in ALSPAC, PIAMA and Generation R



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