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# Roles of Arginase variants, Atopy and Ozone in Childhood Asthma

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## Abstract

**Background—**Arginases (encoded by *ARG1* and *ARG2* genes) may play an important role in asthma pathogenesis through effects on nitrosative stress. Arginase expression is upregulated in asthma and varies with T helper type-2 cytokine levels and oxidative stress.

**Objective—**We aimed to examine whether variants in these genes are associated with asthma, and whether atopy, and exposures to smoking and air pollution influence the associations.

**Methods—**Among non-Hispanic and Hispanic white participants of the Children's Health Study (N=2,946), we characterized variation in each locus (including promoter region) with 6 tagSNPs for *ARG1* and 10 for *ARG2*. Asthma was defined by parental report of physician-diagnosed asthma at study entry.

**Results—**Both *ARG1* and *ARG2* genetic loci were significantly associated with asthma (global locus level p-values=0.02 and 0.04, respectively). Compared to the most common haplotype within each locus, one *ARG1* haplotype was associated with reduced risk (odds ratio (OR) per haplotype copy=0.55; 95% confidence interval (CI): 0.36–0.84) and one *ARG2* haplotype was associated with increased risk (OR per haplotype copy=1.35; 95% CI: 1.04–1.76) of asthma. The effect of the *ARG1* haplotype that was significantly associated with asthma varied by child's history of atopy and ambient ozone ($P_{interaction}$=0.04 and 0.02, respectively). Among atopic children living in high ozone communities, those carrying the *ARG1* haplotype had reduced asthma risk (OR per haplotype copy=0.12; 95% CI: 0.04–0.43; $P_{heterogeneity}$ across atopy/ozone categories=0.008).

**Conclusions—***ARG1* and *ARG2* loci are associated with childhood asthma. The association between *ARG1* variation and asthma may depend on atopy and ambient ozone.

### Keywords

air pollution; asthma genetics; atopy; gene-environment interaction; nitrosative stress

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**Clinical Implications:** Variants in arginase genes are associated with asthma in children. Both genetic (history of atopy) and environmental (ambient ozone) factors could influence the asthma risk associated with arginase variants.

**Capsule Summary:** This paper finds that both arginase I and II genetic loci are significantly associated with asthma occurrence in children. Ambient ozone and child's history of atopy influenced the association between arginase I variants and asthma.

## INTRODUCTION

Both oxidative/nitrosative stress and allergy mediated airway inflammation play important roles in asthma pathogenesis.1 Nitric oxide (NO) is a key mediator of nitrosative stress, 2 as it can react with reactive oxygen species (ROS) to form reactive nitrogen species (RNS, e.g., peroxinitrite). Exhaled NO is also a marker of allergic airway inflammation.3 In addition to its role in airway inflammation, NO is also involved in bronchodilatation and apoptosis. NO is synthesized from L-arginine by nitric oxide synthases (NOS); however, arginase competes with NOS and hydrolyzes L-arginine to urea and ornithine in the urea cycle. Increased arginase activity leads to the increase production of polyamines and proline, which are involved in cell hyperplasia and collagen deposition, respectively. Furthermore, increased arginase activity has been shown to inhibit NO synthesis.4

There are two isoforms of arginase, which are encoded on different chromosomes. Arginase I is encoded by *ARG1* and is located in the cytosol, whereas Arginase II by *ARG2* and is located in mitochondrial matrix.[5] In humans, both isoforms are expressed in airway epithelium, smooth muscle and peribronchial and perivascular connective tissues[6, 7] and in granulocytes but not in macrophages.[8, 9]

A growing body of evidence suggests a role of arginase in asthma occurrence. Asthmatics have significantly higher arginase activity than non-asthmatics.[10] Another study found 3-fold higher arginine levels within airway epithelial cells in asthmatics compared to non-asthmatic controls.[11] Both *ARG1* and *ARG2* gene expressions have been reported to be upregulated in a mouse model of asthma.12 Arginase I protein was highly expressed in bronchoalveolar lavage fluid cells of atopic asthmatics compared to subjects with no atopy/asthma.[12] The role of few single nucleotide polymorphisms (SNPs) in *ARG1* and *ARG2* on asthma occurrence has been reported in only one study to date.[13] Two highly correlated *ARG2* SNPs were associated with asthma but no associations were found for *ARG1* SNPs. It is possible that failure to capture the haplotype diversity in these loci with few SNPs and/or smaller sample size available (n=433) may have limited the opportunity to detect global associations between these genetic loci and asthma.

Based on experimental evidence, associations of arginase variant with asthma may depend upon the airway inflammatory state and exposures that mediate oxidant stress, both of which are strong determinants of asthma. Arginase expression has been shown to be upregulated by interleukin (IL)-4 and IL-13 in murine models of asthma,[12, 14, 15] two of the T helper cell type-2 ($T_H2$) cytokines which have been strongly associated with atopic phenotypes. In addition to $T_H2$ cytokine-mediated allergic airway inflammation, exposures to tobacco smoke and ambient air pollutants result in oxidant stress mediated airway inflammation and have been associated with asthma occurrence and exacerbations. Tobacco smoke has been shown to increase Arginase I expression in airway epithelium among asthmatics compared to non-smoking asthmatics.[6] In this study, Arginase I expression was found in airway smooth muscles in smokers but not in non-smokers. Among ambient air pollutants, exposure to high levels of ozone has been also been shown to produce oxidative stress and inflammation which can modulate NOS isoform expressions.[16] Therefore, it is plausible that the mechanism for the variation of the association of *ARG1* and *ARG2* variants with asthma in children with different ambient air pollutant exposures (e.g., ozone, $PM_{2.5}$, etc) could be in part due to differences in arginase isoform expression.

Based on this evidence, we aimed to test two hypotheses: (1) variations in the *ARG1* and *ARG2* genetic loci are globally associated with childhood asthma; and (2) these associations vary by atopy, tobacco smoke exposures (in utero exposure to maternal smoking and exposure to secondhand smoke in early childhood), and by ambient air pollutants. We tested

these hypotheses in a population-based study conducted among non-Hispanic and Hispanic white children who had participated in the Children's Health Study (CHS).

# METHODS

## Study Design and Participants

The CHS recruited children attending public school in 4th, 7th and 10th grades in 12 southern California communities in 1993 and 1996. The communities were selected primarily on the basis of wide regional variations in ambient pollution levels. At study entry, parents or guardians of each participating student completed a self-administered questionnaire, which included detailed information about demographic and household characteristics as well as information regarding the child's respiratory health. Details about the CHS have been described.[17, 18] The University of Southern California Institutional Review Board reviewed and approved the study. The present analysis included 2,946 children who were either Hispanic (n=936) or Non-Hispanic (n=2,010) whites with known asthma status and complete genotypic data for *ARG1* and *ARG2* single nucleotide polymorphisms (SNPs).

## Assessment of Outcome and Exposures

Children were classified as having asthma if the adult completing the questionnaire reported that a doctor had "ever diagnosed the child as having asthma." Child's atopic status was based on parental report of any allergy and/or hay fever. A child's exposure to maternal smoking *in utero* was based on smoking by biological mother during pregnancy. Secondhand exposure to smoking was based on exposure to smokers inside the house during childhood. Ambient levels of ozone ($O_3$), nitrogen dioxide ($NO_2$), particulate matter with an aerodynamic diameter <10μm ($PM_{10}$) and <2.5μm ($PM_{2.5}$), acid vapor and elemental and organic carbon were measured at air monitoring sites in each of the 12 communities from 1992 onwards as previously described.[17, 18] Based on the median of average pollutant concentrations across the study communities, we categorized each exposure into high and low pollutant levels. Because $PM_{10}$, $PM_{2.5}$, $NO_2$, acid vapor, and elemental and organic carbon were highly correlated and ozone was not significantly correlated with the former pollutants (see Table E1 in the Online Repository), and communities defined as "high" or "low" pollution categories based on any of the non-ozone pollutants were the same for all of the correlated pollutants, we only examined whether the associations between *ARG1* and *ARG2* haplotypes and asthma varied by ozone and $PM_{2.5}$.

## SNP Selection and Genotyping

In representative non-Hispanic and Hispanic white samples from the Multiethnic Cohort (n~71 each),[19] 1–3 SNPs/kb were genotyped using the Illumina Golden Gate Assay to determine ethnic-specific minor allele frequencies (MAFs) and patterns of linkage disequilibrium (LD) around 20kb upstream and 10kb downstream region unless another gene was located within this region. Minimum set of haplotype-tagged SNPs (htSNPs) with MAFs ≥0.05 were chosen to explain >90% of haplotype diversity ($R^2h \geq 0.90$) for each haplotype block using the TagSNPs program (available at http://www-rcf.usc.edu/~stram/tagSNPs.html).[20] Redundant tagSNPs were genotyped to substitute for critical SNPs in the event of assay failure in the latter. SNPs were genotyped using Illumina BeadArray platform. Based on these criteria, we selected 6 SNPs in *ARG1* and 10 SNPs in *ARG2* for our analyses (Table I). These SNPs had call rates >90%. Details of buccal sample collection and quality control protocol are presented in the online supplement.

### Haplotype Estimation

For each gene, high multiallelic D′ between haplotype blocks allowed estimating common haplotypes (>5% frequency in either ethnic group) across using all SNPs within each locus. Haplotype frequencies of unphased *ARG1* and *ARG2* SNPs were estimated separately for Hispanic and non-Hispanic white subjects using a SAS macro code available with the TagSNPs program. This haplotype estimation technique provides the maximum likelihood estimates of the haplotype frequencies assuming Hardy-Weinberg equilibrium.[20]

### Statistical analysis

Initially, the global association between each genetic locus and asthma was determined using haplotypes and principal components (PC) analyses[21] in the overall sample adjusting for ethnicity and other potential confounders. If the locus was significantly associated with asthma, ethnic-specific analyses were conducted to examine whether the results varied by ethnicity. Subsequently, to further investigate the global associations, we conducted SNP-based analyses to determine whether specific SNPs could account for the locus-level significance. To address multiple comparison issues across correlated SNPs, we adjusted the p-values for significant SNP-based results at locus level using the p_ACT procedure (available at http://csg.sph.umich.edu/boehnke/p_act.php).[22] This procedure has been shown to provide valid adjustment of p-values for multiple testing similar to the permutation test but uses significantly less computational time. Finally, modifying effects of atopy, tobacco smoke and air pollution exposures on the associations between risk haplotypes and asthma were evaluated.

We used additive genetic model for the SNPs for the PC- and SNP-based analyses and for haplotypes for haplotype-based analyses. In the PC-based approach, PCs (ordered by magnitude of explained variance) that explained at least 80% of the SNP variance within the locus was used to test locus-level significance. This approach, which has been assessed previously,[21] captures the underlining linkage disequilibrium and correlations among the SNPs within the locus. In the haplotype-based approach, uncommon haplotypes (frequencies <0.05 in both ethnic groups) were combined into an “Other haplotypes” category.

Logistic regression models were fitted to compute odds ratios (ORs) and 95% confidence intervals (CIs). Age, sex, ethnicity, child’s history of atopy, parental asthma, parent/guardian’s education, *in utero* exposure to maternal smoking, health insurance coverage, and community of residence were considered as potential confounders. Modifying effects of tobacco smoke exposures, ambient air pollutants and atopy on the associations between haplotypes and asthma were evaluated using likelihood ratio tests with appropriate interaction terms. All tests were two-sided at a 5% significance level. We used R program to implement the p_ACT procedure. The rest of the analyses were done with SAS version 9.1 (SAS Institute Inc, Cary, NC).

## RESULTS

There were limited differences in minor allele and haplotype frequencies by ethnicity (Table I; for SNP location and distance between SNPs, see Table E2 in the Online Repository). Haplotype block structure was similar for *ARG1* by ethnicity (see Figure E1 in the Online Repository) but less so for *ARG2* (see Figure E2 in the Online Repository). For *ARG1*, common haplotypes accounted for 92% of the haplotype frequencies in both ethnic groups. For *ARG2*, 86% and 82% cumulative haplotype frequencies were obtained with common haplotypes for non-Hispanic and Hispanic white, respectively.

Boys were at higher asthma risk than girls (Table II). Atopy and parental asthma were positively associated with asthma. Children with asthma were more likely to have health

insurance compared to children without asthma. The prevalences of asthma in Non-Hispanic and Hispanic white children were comparable (15.7% vs. 14.3%; *P*-value = 0.32).

### *ARG1* and Asthma

Variations in the *ARG1* locus was globally associated with asthma (Global *P*-value=0.04; Table III) in the haplotype-based approach but not in the PC-based approach (See Table E3 in the Online Repository). In addition, no *ARG1* SNP was associated with asthma (Table E3). These findings suggest that a haplotype rather than SNPs may underlie the association with the locus. Compared to the most common *ARG1* haplotype that carried the wild-type allele for all studied SNPs, the haplotype that carried the variant allele for RS2749935 (i.e., *ARG1h4* haplotype) was associated with a 45% reduced risk of asthma (OR=0.55; 95% CI: 0.36–0.84). These associations did not vary substantially by ethnicity.

### *ARG2* and Asthma

Variations in the *ARG2* locus was globally associated with asthma in both haplotype (Global *P*-value=0.02; Table III) and PC-based (Global *P*-value=0.01; Table E3 in the Online Repository) analyses. Each copy of *ARG2h3* haplotype was associated with 35% increased asthma risk (95% CI: 1.04–1.76) compared to the most common haplotype. Furthermore, *ARG2h6* haplotype was associated with a 50% reduced asthma risk (OR=0.50; 95% CI: 0.28–0.92) in non-Hispanic whites only. However, the *ARG1* and *ARG2* haplotype-specific ORs did not vary by ethnicity (both *P*-values for interaction>0.66). In SNP-based analysis, 4 of the 10 *ARG2* SNPs were associated with asthma in the combined population (Table E3). After adjusting for multiple comparisons, each variant allele of RS3742879 was associated with 31% increased asthma risk (adjusted p-value=0.007).

### Modifying Role of Atopy, Smoking, and Ambient Air Pollutants

Atopy and ambient ozone modified the association between *ARG1h4* haplotype and asthma (*P*-values for interaction=0.04 and 0.02, respectively Table IV), but did not influence the relationship of *ARG2h6* haplotype and asthma. None of the SNPs or haplotypes in *ARG1* and *ARG2*, however, was associated with atopy. In utero and secondhand smoke exposures and ambient $PM_{2.5}$ did not modify the associations between *ARG1* and *ARG2* haplotypes and asthma. Because two SNPs in *ARG2* (RS4902503 and RS3742879) showed significant associations (Table E3), we further evaluated whether the associations of these SNPs with asthma varied by atopy, tobacco smoke or air pollution exposures. None of these factors influenced the associations of these SNPs with asthma (not shown).

Among atopic children, each copy of *ARG1h4* haplotype was associated with reduced asthma risk (OR=0.35; 95% CI: 0.19–0.66); however this haplotype was not associated with asthma among non-atopic children. Among children living in high ozone communities (annual average >50ppb), each copy of *ARG1h4* haplotype was associated with reduced asthma risk (OR=0.31; 95% CI: 0.16–0.63). In contrast, no association was found between *ARG1h4* haplotype and asthma in children living in communities with low ambient ozone levels.

We found significant heterogeneity in the relationship of *ARG1h4* haplotype with asthma across joint categories of atopy and ozone exposure (P-value for heterogeneity=0.008; Table V). Among children with a history of atopy who lived in communities with high ambient ozone concentration, each copy of *ARG1h4* haplotype was associated with greatly reduced asthma risk (OR=0.13; 95% CI: 0.04–0.44). Non-atopic children living in high ozone communities or atopic children living in low ozone communities had reduced asthma risk but the ORs were not statistically significant.

### Gene-Gene interaction

We did not find any significant gene-gene interaction between *ARG1* and *ARG2* haplotypes or SNPs and asthma (not shown). Both *ARG1* and *ARG2* haplotypes showed independent effects on asthma. In a model that contained both these haplotypes along with other covariates, compared to the other haplotypes within each locus, the ORs for asthma with each copy of *ARG1h4* and *ARG2h3* haplotypes were 0.54 (95% CI: 0.36–0.83) and 1.34 (95% CI: 1.06–1.69), respectively.

### Sensitivity analyses

Previously, we have reported associations with variants in genes in the oxidant stress pathway (i.e., *GSTP1 Ile*105*Val*,[23] *GSTM1* null,[24] *TNF* -308G/A,[25] short allele of *HMOX1*,26 and catalase -262C>T26) and asthma/wheeze in this cohort. In sensitivity analyses, we adjusted our models with these variants, and found that the *ARG1* and *ARG2* haplotypes and SNPs had independent effects (see Table E4 in the Online Repository). Restricting the analysis to children with health insurance did not affect any observed associations (not shown).

## DISCUSSION

Using data from a large, well-characterized population-based study, we observed significant associations between *ARG1* and *ARG2* genetic loci and asthma in children. We showed that effect of *ARG1* variants on asthma could represent a phase (haplotypic) effect. Furthermore, we found that both genetic (history of atopy) and environmental (ambient ozone) factors influenced the asthma risk associated with *ARG1* but not with *ARG2* variants. This is a novel finding that suggests that atopy and ambient ozone could influence the expression of these two genes differently.

Epidemiologic evidence of associations of these genetic loci with asthma is limited. Only one study to date has reported associations between two highly correlated *ARG2* SNPs ($R^2$=0.937) and asthma among Mexican children without adjusting the p-values for multiple comparisons.[13] One advantage of our study was the selection of more SNPs with greater coverage of the promoter region (20kb upstream). This SNP selection approach allowed better identification of haplotype diversity and enabled us to evaluate the global association of each locus with asthma.

Very limited data exist on the functional significance of *ARG1* and *ARG2* SNPs. We were unable to find any published work on the functional effects of *ARG1* and *ARG2* variants. According to the Single Nucleotide Polymorphism database (dbSNP), there are 3 non-synonymous and 1 synonymous SNPs in *ARG1* and 4 non-synonymous and 2 synonymous SNPs in *ARG2*. However, the MAFs of these SNPs are ≤1% in whites. Given the paucity of functional data, we hypothesize that the studied genetic variants or other “causal” SNPs linked with these SNPs may affect asthma risk through nitrosative stress mediated airway hyperresponsiveness (AHR) and/or remodeling. We have identified several putative transcription factor binding sites on some of the *ARG1* and *ARG2* SNPs in the 5′ genomic regions and in introns (see Table E5 in the Online Repository). These transcription factors have diverse functions on lung development, morphogenesis, and immune functions.[27–30] Whether these SNPs with transcription factors binding sites affect arginase expression in the airways or leukocytes remains to be explored.

There are several potential mechanisms by which arginase could affect asthma risk. Arginase is a key enzyme in the urea cycle. In the final step of the cycle, arginase hydrolyzes L-arginine to produce ornithine and urea. Arginase and NOS competes for the common substrate L-arginine. Increase arginase activity increases the production of

ornithine, which can be converted into proline and polyamines in the mitochondrial matrix. Proline is a component of collagen and mucus and polyamines increase cell proliferation. Therefore, it is plausible that increased collagen deposition and cell proliferation due to high arginase activity could lead to airway remodeling – a pathophysiologic hallmark of asthma.

Beside the effects mediated by proline and polyamines, arginase over-expression affect NF-κB activity,[4] RNS production,[31, 32] and respiratory physiology.[33–35] Activation of transcription factor NF-κB has been implicated in asthma,[36] and NF-κB inhibition attenuated allergic airway inflammation, AHR and reduced $T_H2$ cytokine synthesis in a mouse model.37 Reduced L-arginine due to increased arginase activity could uncouple NOS and result in increased formation of peroxynitrite31, [32]- a highly reactive nitrogen species which may promote airway inflammation. In addition, increased arginase activity has been associated with increased airway hyperresponsiveness (AHR), reduced airway smooth muscle relaxation and increased airway remodeling in animal models.[33–35] Furthermore, arginase inhibition promptly reversed allergen-induced AHR and airway inflammation *in vivo* in a guinea-pig model.[38] In light of these observations and our findings, we speculate that the *ARG1* protective and *ARG2* risk haplotypes that were significantly associated with asthma could reduce and increase arginase activity, respectively relative to the other haplotypes. This hypothesis needs to be examined further.

We found that atopy and ozone modified the relationship of *ARG1* haplotypes but did not affect the association of *ARG2* haplotypes with asthma. Existing data show that these two isoforms have differential expression profiles. In experimental studies, $T_H2$ cytokines (IL-4 and IL-13) promoted expression of *ARG1* but not of *ARG2*.[39, 40] This differential expression pattern may explain our findings of a modifying role of atopy on the effect of *ARG1* haplotype on asthma but no such modulation for *ARG2* haplotypes. Although ozone imparts oxidant stress mediated injury to the airways, we are unaware of any published report that ozone could modulate arginase expression. However, ozone exposure induces inducible NOS (iNOS) in alveolar macrophages and type II alveolar epithelium in animals.[41] If ozone mediated upregulation of iNOS reduces L-arginine availability for *ARG1*, then it is plausible that the arginase activity could vary across *ARG1* haplotypes with ozone exposure. This could be one explanation for finding a stronger reduced risk in children carrying one *ARG1* haplotype compared to those not carrying that haplotype among children who lived in high ozone communities. Further research is warranted to examine the effect of ozone exposure on arginase expression.

We considered several potential sources of errors and biases in this study. Children who participated in the genetic study of the CHS by providing buccal samples differed modestly on demographic factors (age, sex, and ethnicity), socioeconomic (SES) factors (family income, parental education, and health insurance coverage), and smoking exposures (in utero and childhood) from those who did not participate. We compared models with and without each covariate of interest to determine the impact of these factors on the risk estimates. None of these covariates changed the ORs by more than 5%. We used parental report of physician-diagnosed asthma in our study and concern has been raised in that parental report may not reflect physician diagnosis and access to healthcare may lead to variation in likelihood of diagnosis. To investigate this potential bias, we reviewed medical records of children with asthma and found strong evidence that parental-report reflected physician diagnosis.[42] Furthermore, restricting our analyses to children with health insurance yielded similar results (not shown). We acknowledge that our atopy definition based on parental report of allergy and/or hay fever could be misclassified, but it is unlikely to be differential with respect to the genotypes and ambient ozone exposure.

In association studies involving multiethnic populations, population stratification could bias the results. To address this potential bias, we have excluded and not genotyped children who were African-American (N=163), Asian (N=166) or belonged to a mixed ethnic background (N=192) because of insufficient sample sizes to conduct ethnic-specific analysis. Because our results for the genetic associations yielded similar results by ethnicity, our analytic approach of conducting pooled analyses adjusting for ethnicity appear reasonable. In addition, restricting the analyses to non-Hispanic whites, which provided larger sample size for ethnic-specific associations, provided similar results. Because we could not generalize our finding to African-American or Asian populations, further investigation is warranted in these populations.

To conclude, we found that both *ARG1* and *ARG2* genetic loci are associated with childhood onset asthma. The effect of *ARG1* haplotypes on asthma may be modulated by atopy and exposure to ambient ozone. Arginase has been considered a candidate gene for asthma; however, limited studies in humans have been conducted. Further research is warranted to resequence the loci to identify novel variants and then determine the impact of those variants on arginase activity, airway inflammation and AHR in human airways. This could explain the possible mechanism(s) by which variants in these genes could affect asthma risk in children.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

| | |
|---|---|
| *ARG1* | arginase I gene |
| *ARG2* | arginase II gene |
| AHR | airway hyperresponsiveness |
| CHS | Children's Health Study |
| CI | confidence interval |
| dbSNP | Single Nucleotide Polymorphism database |
| GSTP1 | glutathione s-transferase P1 gene |
| GSTM1 | glutathione s-transferase M1 gene |
| htSNPs | haplotype-tagged SNPs |

| | |
|---|---|
| HMOX1 | heme oxygenase gene |
| IL | interleukin |
| LD | linkage disquilibrium |
| MAF | minor allele frequencies |
| NO | nitric oxide |
| NOS | nitric oxide synthase |
| $NO_2$ | nitrogen dioxide |
| OR | odds ratio |
| $O_3$ | ozone |
| PC | principal component |
| $PM_{2.5}$ | particulate matter with an aerodynamic diameter <2.5μm |
| $PM_{10}$ | particulate matter with an aerodynamic diameter <10μm |
| RNS | reactive nitrogen species |
| ROS | reactive oxygen species |
| SES | socioeconomic status |
| SNP | single nucleotide polymorphism |
| $T_H2$ | helper T lymphocyte type-2 |
| TNF | tumor necrosis factor gene |

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**TABLE I**

Minor allele and haplotype frequencies of *ARG1* and *ARG2* by ethnicity

| Gene, SNPs | Alleles* | Minor allele Frequency | | Gene, Haplotypes† | Haplotype frequency | |
|---|---|---|---|---|---|---|
| | | Non- Hispanic white | Hispanic white | | Non- Hispanic white | Hispanic white |
| ***ARG1*** | | | | ***ARG1*** | | |
| RS2608981 | A/C | 0.35 | 0.22 | 000000 (*ARG1h1*) | 0.57 | 0.43 |
| RS3895535 | G/A | 0.25 | 0.18 | 111111 (*ARG1h2*) | 0.13 | 0.21 |
| RS2608937 | G/A | 0.25 | 0.17 | 000110 (*ARG1h3*) | 0.11 | 0.08 |
| RS2749935 | A/T | 0.49 | 0.38 | 000100 (*ARG1h4*) | 0.05 | 0.05 |
| RS2781659 | A/G | 0.41 | 0.32 | 100110 (*ARG1h5*) | 0.04 | 0.10 |
| RS2246012 | T/C | 0.27 | 0.16 | 000001 (*ARG1h6*) | 0.02 | 0.05 |
| | | | | Other haplotypes‡ | 0.08 | 0.08 |
| ***ARG2*** | | | | ***ARG2*** | | |
| RS12885261 | C/T | 0.41 | 0.59 | 1000100000 (*ARG2h1*) | 0.28 | 0.16 |
| RS7144243 | A/G | 0.17 | 0.21 | 0000000010 (*ARG2h2*) | 0.16 | 0.16 |
| RS3759757 | G/C | 0.36 | 0.26 | 1000000010 (*ARG2h3*) | 0.13 | 0.08 |
| RS4902501 | T/C | 0.35 | 0.26 | 0000000100 (*ARG2h4*) | 0.12 | 0.11 |
| RS7156352 | C/T | 0.19 | 0.31 | 1111011101 (*ARG2h5*) | 0.09 | 0.05 |
| RS4902503 | A/G | 0.42 | 0.26 | 0111010100 (*ARG2h6*) | 0.04 | 0.05 |
| RS7140310 | T/G | 0.16 | 0.14 | 0011010101 (*ARG2h7*) | 0.04 | 0.15 |
| RS742869 | G/A | 0.56 | 0.40 | 0000011101 (*ARG2h8*) | 0.003 | 0.06 |
| RS3742879 | A/G | 0.25 | 0.30 | Other haplotypes‡ | 0.14 | 0.18 |
| RS10483801 | C/A | 0.31 | 0.19 | | | |

*Alleles are presented as major/minor alleles. For SNP location and distance between SNPs, see Table E2.

†SNP order in *ARG1* haplotypes is RS2608981-RS3895535-RS2608937-RS2749935-RS2781659-RS2246012 and in *ARG2* haplotypes is RS12885261-RS7144243-RS3759757-RS4902501-RS7156352-RS4902503-RS7140310-RS742869-RS3742879-RS10483801. Within each haplotype, '0' and '1' represents wild-type and variant alleles at the ordered SNP position, respectively. Each haplotype was abbreviated with the gene name appearing first followed by a number.

‡Haplotypes with <5% frequencies are combined into the "other haplotypes" category.

**TABLE II**

Descriptive statistics of the study population.*

| | No asthma | | Asthma | | P-value‡ |
|---|---|---|---|---|---|
| | N† | (%) | N† | (%) | |
| Sex | | | | | |
| Girls | 1376 | (55.1) | 201 | (44.7) | <0.0001 |
| Boys | 1120 | (44.9) | 249 | (55.3) | |
| Age (years) | | | | | |
| ≤ 10 | 1398 | (56.1) | 245 | (54.4) | 0.45 |
| 11–12 | 468 | (18.7) | 79 | (17.6) | |
| > 12 | 630 | (25.2) | 126 | (28.0) | |
| Ethnicity | | | | | |
| Non-Hispanic white | 1694 | (67.9) | 316 | (70.2) | 0.32 |
| Hispanic white | 802 | (32.1) | 134 | (29.8) | |
| Parental history of asthma | | | | | |
| No | 1950 | (82.8) | 247 | (57.8) | <0.0001 |
| Yes | 405 | (17.2) | 180 | (42.2) | |
| Exposure to maternal smoking *in utero* | | | | | |
| No | 2024 | (82.9) | 357 | (80.2) | 0.17 |
| Yes | 418 | (17.1) | 88 | (19.8) | |
| Exposure to secondhand smoke | | | | | |
| No | 1710 | (70.9) | 292 | (66.5) | 0.06 |
| Yes | 700 | (29.1) | 147 | (33.5) | |
| Child's atopic status** | | | | | |
| No | 1582 | (68.0) | 127 | (31.7) | <0.0001 |
| Yes | 743 | (32.0) | 274 | (68.3) | |
| Annual family income (in US dollars) | | | | | |
| < $15,500 | 303 | (14.1) | 48 | (12.1) | 0.09 |
| $15,000 – $49,999 | 919 | (42.8) | 154 | (38.9) | |
| ≥ $50,000 | 924 | (43.1) | 194 | (49.0) | |

| | No asthma | | Asthma | | P-value‡ |
|---|---|---|---|---|---|
| | N† | (%) | N† | (%) | |
| Parent/guardian education | | | | | |
| < 12th grade | 313 | (12.9) | 30 | (6.7) | 0.002 |
| 12th grade | 459 | (18.9) | 87 | (19.6) | |
| Some college | 1092 | (45.0) | 232 | (52.1) | |
| College | 246 | (10.1) | 44 | (9.9) | |
| Some graduate | 315 | (13.0) | 52 | (11.7) | |
| Health insurance coverage | | | | | |
| No | 378 | (15.4) | 35 | (7.9) | <0.0001 |
| Yes | 2078 | (84.6) | 406 | (92.1) | |

*Non-Hispanic and Hispanic white children in the Children's Health Study with complete data on *ARG1* and *ARG2* SNPs were included. For details, see the methods.

†Numbers always do not add up because of missing data.

‡P values from Pearson chi-square tests comparing children with asthma with those without asthma.

**Child's atopic status was determined based on parental report of allergy and hay fever.

**TABLE III**

Associations between *ARG1* and *ARG2* haplotypes and asthma

| Gene, haplotypes | Non-Hispanic white | Hispanic white | Combined |
|---|---|---|---|
| | OR (95% CI)* | OR (95% CI)* | OR (95% CI)* |
| ***ARG1* haplotypes**† | | | |
| *ARG1h1* | 1.0 (Reference) | 1.0 (Reference) | 1.0 (Reference) |
| *ARG1h2* | 0.96 (0.72–1.28) | 0.85 (0.56–1.28) | 0.93 (0.74–1.17) |
| *ARG1h3* | 1.00 (0.74–1.36) | 1.23 (0.76–1.98) | 1.06 (0.82–1.37) |
| *ARG1h4* | **0.61 (0.37–1.00)** | **0.41 (0.17–0.97)** | **0.55 (0.36–0.84)** |
| *ARG1h5* | 1.33 (0.85–2.09) | 1.01 (0.57–1.77) | 1.22 (0.87–1.72) |
| *ARG1h6* | 0.83 (0.38–1.81) | 0.57 (0.23–1.38) | 0.69 (0.39–1.21) |
| Other haplotypes | 0.96 (0.66–1.39) | 0.99 (0.55–1.77) | 0.95 (0.70–1.29) |
| **Global P-value**‡ | 0.39 | 0.18 | **0.04** |
| ***ARG2* haplotypes**† | | | |
| *ARG2h1* | 1.0 (Reference) | 1.0 (Reference) | 1.0 (Reference) |
| *ARG2h2* | 1.30 (0.97–1.73) | 1.10 (0.67–1.82) | 1.22 (0.96–1.56) |
| *ARG2h3* | **1.38 (1.02–1.87)** | 1.28 (0.73–2.26) | **1.35 (1.04–1.76)** |
| *ARG2h4* | 1.00 (0.72–1.39) | 0.89 (0.50–1.61) | 0.98 (0.74–1.30) |
| *ARG2h5* | 0.86 (0.58–1.27) | 0.68 (0.31–1.51) | 0.83 (0.59–1.18) |
| *ARG2h6* | **0.50 (0.28–0.92)** | 1.28 (0.60–2.71) | 0.73 (0.47–1.15) |
| *ARG2h7* | 0.74 (0.41–1.32) | 0.96 (0.57–1.63) | 0.88 (0.61–1.26) |
| *ARG2h8* | 0.80 (0.09–7.18) | 0.87 (0.41–1.86) | 0.79 (0.40–1.54) |
| Other haplotypes | 1.37 (0.99–1.91) | 1.19 (0.71–2.00) | 1.30 (0.99–1.71) |
| **Global P-value**‡ | **0.007** | 0.89 | **0.02** |

*ORs are calculated using the most common haplotype as reference and represent the risk associated with per copy of the haplotype. ORs are adjusted for age, sex, ethnicity (for the combined population), child's atopic status, parental history of asthma, parent/guardian's education, *in utero* exposure to maternal smoking, exposure to secondhand smoke, health insurance coverage, and community of residence.

†For the order of SNPs in haplotypes, see the footnote of Table I.

‡Global P-values are from likelihood ratio tests compared to a model with no haplotype data and were based on 6df for *ARG1* and 8df for *ARG2*.

## TABLE IV

Associations between *ARG1* and *ARG2* risk haplotypes and asthma and modifying effects of atopy, smoking exposures, and air pollution.

| **Modifying factors** | ***ARG1h4* haplotype** | ***ARG2h3* haplotype** |
|---|---|---|
| | **OR (95% CI)*** | **OR (95% CI)*** |
| Marginal effects | **0.55 (0.36–0.83)** | **1.34 (1.06–1.68)** |
| Children's atopy status | | |
| No atopy (n=1709) | 0.87 (0.45–1.66) | 1.38 (0.95–2.00) |
| Atopy (n=1017) | **0.35 (0.19–0.66)** | 1.17 (0.84–1.62) |
| P-value† | **0.04** | 0.61 |
| In utero exposure to maternal smoking | | |
| No (n=2381) | **0.53 (0.32–0.88)** | 1.29 (0.99–1.67) |
| Yes (n=506) | 0.76 (0.33–1.76) | 1.37 (0.78–2.42) |
| P-value† | 0.73 | 0.74 |
| Exposure to secondhand smoke in childhood | | |
| No (n=2002) | **0.53 (0.31–0.90)** | 1.23 (0.92–1.64) |
| Yes (n=847) | 0.67 (0.33–1.36) | **1.75 (1.16–2.64)** |
| P-value† | 0.81 | 0.35 |
| Ambient Ozone Categories | | |
| Low (n=1420) | 0.90 (0.52–1.55) | 1.35 (0.96–1.90) |
| High (n=1526) | **0.31 (0.16–0.63)** | 1.36 (0.99–1.86) |
| P-value† | **0.02** | 0.96 |
| Ambient $PM_{10}$ Categories | | |
| Low (n=1545) | 0.66 (0.38–1.15) | 1.36 (0.99–1.87) |
| High (n=1401) | **0.44 (0.22–0.88)** | 1.32 (0.94–1.86) |
| P-value† | 0.36 | 0.91 |

*The ORs for marginal effect represent asthma risk associated with per copy of the haplotype. The rest of the ORs represent asthma risk associated with per copy of the haplotype within each stratum of modifying factors. These ORs are adjusted for age, sex, ethnicity, child's atopic status, parental history of asthma, parent/guardian's education, *in utero* exposure to maternal smoking, exposure to secondhand smoke, health insurance coverage, and community of residence.

†P-values for interactions were obtained from likelihood ratio tests and were based on 1df.

## TABLE V

Association of *ARG1h4* haplotype haplotype with asthma within strata of atopy and ambient ozone.

| Children's atopy status | Ambient Ozone Categories | N* | *ARG1h4* haplotype OR (95% CI) † | P-value‡ |
|---|---|---|---|---|
| Non-atopic | Low | 59/723 | 1.30 (0.57–2.97) | **0.008** |
| Non-atopic | High | 68/859 | 0.55 (0.19–1.57) | |
| Atopic | Low | 145/399 | 0.66 (0.31–1.40) | |
| Atopic | High | 129/344 | **0.12 (0.04–0.43)** | |

*N represents numbers of children with/without asthma within each atopy and ambient ozone categories.

†ORs represent asthma risk associated with per copy of *ARG1h4* haplotype within each atopy and ambient ozone categories. The ORs are adjusted for age, sex, ethnicity, parental history of asthma, parent/guardian's education, *in utero* exposure to maternal smoking, exposure to secondhand smoke, health insurance coverage, and community of residence.

‡P-value for heterogeneity of haplotype effects across categories of atopy/ozone and was obtained from a likelihood ratio test and was based on 3df.