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Genetic polymorphisms in arginase I and II and childhood asthma and atopy

Huiling Li, MD[1], **Isabelle Romieu, MD**[2], **Juan-Jose Sienra-Monge, MD**[3], **Matiana Ramirez-Aguilar, MD**[2], **Blanca Estela del Rio-Navarro, MD**[3], **Emily O. Kistner, PhD**[1], **Håkon K. Gjessing, PhD**[4], **Irma del Carmen Lara-Sanchez, BS**[2], **Grace Y. Chiu, PhD**[5], and **Stephanie J. London MD**[1]

1 *Laboratory of Respiratory Biology (HL, SJL), Epidemiology Branch (SJL) and Biostatistics Branch (EOK), Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC*

2*National Institute of Public Health, Cuernavaca, Morelos, Mexico*

3*Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico*

4*Norwegian Institute of Public Health, Oslo, Norway*

5*Westat, Inc, Research Triangle Park, NC.*

Abstract

Background—A recent microarray study implicated arginase I (*ARG1*) and arginase II (*ARG2*) in mouse allergic asthma models and human asthma.

Objectives—To examine the association between genetic variation in *ARG1* and *ARG2* and childhood asthma and atopy risk.

Methods—We enrolled 433 case-parent triads, consisting of asthmatics 4 to 17 years and their biologic parents, from the allergy clinic of a public hospital in Mexico City between 1998 and 2003. Atopy to 24 aeroallergens was determined by skin prick tests. We genotyped 4 single nucleotide polymorphisms (SNPs) of *ARG1* and 4 SNPs of *ARG2* with minor allele frequencies over 10% using the TaqMan assay.

Results—*ARG1* SNPs and haplotypes were not associated with asthma but all four *ARG1* SNPs were associated with the number of positive skin tests (P = 0.007 to 0.018). Carrying two copies of minor alleles for either of two highly associated *ARG2* SNPs was associated with a statistically significant increased relative risk (RR) of asthma [1.5, 95% confidence interval (CI) 1.1–2.1 for arg2s1; RR = 1.6, 95% CI = 1.1–2.3 for arg2s2]. The association was slightly stronger among children with a smoking parent (arg2s1 RR = 2.1, 95% CI = 1.2 – 3.9 with a smoking parent; RR =1.2, 95% CI = 0.8–1.9 without, interaction P = 0.025). Haplotype analyses reduced the sample size but supported the single SNP results. One *ARG2* SNP was related to the number of positive skin tests (P = 0.027).

Corresponding Author: Stephanie J. London, MD, DrPH, National Institute of Environmental Health Sciences, PO Box 12233, MD A3-05, Research Triangle Park, NC 27709, USA, Phone: (919) 541-5772, FAX: (919) 541-2511, E-mail: london2@niehs.nih.gov.

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Capsule Summary

A microarray study and mechanistic studies implicated arginase I and arginase II in mouse allergic asthma models and human asthma. Our study indicated the polymorphisms in arginase I and arginase II may contribute to atopy and asthma.

Conclusions—Variation in arginase genes may contribute to asthma and atopy in children.

Keywords

ARG1; ARG2; “genetic predisposition to disease”; SNP; “polymorphism; single nucleotide”; “respiratory hypersensitivity”; “skin tests”; asthma; “tobacco smoke pollution”

Abbreviations

ARG1 = arginase I; ARG2 = arginase II; CI = confidence interval; D′ = Lewontin’s standardized disequilibrium coefficient; eNOS = endothelial nitric oxide synthase; IgE = immunoglobulin E; IL-13 = interleukin-13; IL-4 = interleukin-4; LD = linkage disequilibrium; NO = nitric oxide; NOS = nitric oxide synthase; r^2 = squared correlation coefficient; RR = relative risk; SNP = single nucleotide polymorphism; STAT6 = signal transducer and activator of transcription 6; TDT = transmission disequilibrium test; Th2= T helper lymphocytes type 2

INTRODUCTION

Candidate genes for asthma have arisen from linkage studies as well as knowledge of mechanisms involved in the pathogenesis of asthma. Recently, microarray analyses have identified novel asthma candidate genes[1]. In two murine asthma models, Zimmermann *et al.* identified increased expression of genes coding for two arginase isoforms, arginase I (*ARG1*), and arginase II (*ARG2*) in lung tissue[1]. Further, *ARG1* expression was increased in lung macrophages from peribronchial and perivascular inflammatory sites in affected mice and in bronchoalveolar lavage cells from asthmatic humans. *ARG1* mRNA was strongly detected in human asthmatic lung biopsy tissue with predominant localization to submucusal inflammatory cells as well as epithelial cells.

Arginases may contribute to asthma pathogenesis through various mechanisms, including inhibition of nitric oxide (NO) generation, reduced arginine bioavailability, and increased ornithine production, which could lead to airway remodeling due to altered polyamine and proline synthesis[2, 3]. Of note, *ARG2* lies close to an asthma linkage region on chromosome 14q24[4].

Based on the increasing evidence for a role of arginases in asthma pathogenesis [2, 5], we investigated single nucleotide polymorphisms (SNPs) in *ARG1* and *ARG2* in relation to asthma risk in a case-parent triad study of childhood asthma in Mexico City.

METHODS

Study Design and Subject Enrollment

Using the case-parent triad design[6, 7], we recruited nuclear families consisting of a case and both parents. Cases were children aged 4–17 years with asthma, diagnosed by a pediatric allergist at the allergy referral clinic of a large public pediatric hospital in central Mexico City (Hospital Infantil de Mexico Federico Gomez), between June 1998 and November 2003. Children and parents provided blood samples as sources of DNA. We enrolled 433 complete case-parent triads.

A pediatric allergist based the diagnosis of asthma on clinical symptoms and response to treatment[8]. The severity of asthma among the cases was rated by a pediatric allergist according to symptoms in the Global Initiative on Asthma schema as mild (intermittent or persistent), moderate and severe[9]. Pulmonary function was measured using the EasyOne spirometer (ndd

Medical Technologies, Andover, MA, USA) for 332 subjects according to ATS specifications[10]. The best test out of three technically acceptable tests was selected. Spirometric prediction equations from a Mexico City childhood population were used to calculate the percent predicted forced expiratory volume in one second (FEV_1)[11]. Children were asked to hold asthma medications on the morning of the test.

Atopy was determined using skin prick test. The following battery of 24 aeroallergens common in Mexico City was used: Aspergillus fumigatus, Alternaria, mucor, Blattella germanica, Periplaneta americana, Penicillium, cat, dog, horse, Dermatophagoides (pteronyssinus and farina), Ambrosia, Artemisia ludoviciana, Cynodon dactylon, Chenopodium album, Quercus, Fraxinus, Helianthus, Ligustrum vulgare, Lolium perenne, Plantago lanceolata, Rumex crispus, Schinus molle, Salsola, and Phleum pratense. Histamine was used as positive control and glycerin as negative control. Children were considered atopic if the diameter of the skin reaction to at least one allergen exceeded 4 mm. The test was considered valid if the reaction to histamine was >= 6 mm according to the grading of skin prick test recommended by Aas and Berlin[12]. Skin test data were available on 420 (of 433) triads with complete genotyping data.

The Institutional Review Boards of the Mexican National Institute of Public Health, the Hospital Infantil de Mexico Federico Gomez and the U.S. National Institute of Environmental Health Sciences approved the protocol. Parents provided consent for the child's participation.

SNP Selection

To select SNPs in *ARG1* and *ARG2*, we searched the dbSNP Variation Database from NCBI LocusLink (http://www.ncbi.nlm.nih.gov/SNP) and Applied Biosystem SNP genotyping products (http://www.appliedbiosystems.com/catalog). Because there is no functional data for arginase SNPs and because complete resequencing has not been done in our Mexican population, nor others, we selected SNPs based on adequate minor allele frequency (at least 10%) and spacing throughout the gene. We selected 4 SNPs spaced at 1–3kb, including 3kb upstream genomic region of gene *ARG1* of which the genomic size is about 11kb. For *ARG2*, we selected 4 SNPs with 10–25kb interval including 10kb upstream and 5kb downstream of genomic region of the gene. The genomic size of *ARG2* is about 32kb.

Genotyping of ARG1 and ARG2 SNPs

Peripheral blood lymphocytes were isolated, and DNA was extracted using Gentra Puregene kits (Gentra Systems, Minneapolis, MN). Primers and probes were purchased from Assay-by-Design and Assay-on-Demand (Applied Biosystems, Foster City, CA). All PCR amplifications were performed using the 5′-nuclease assay on GeneAmp PCR Systems 9700. Details of the genotyping methods are included in the Journal's Online Repository.

We ascertained nonparentage with a set of short-tandem repeats (AmpFLSTR Profiler Plus; Applied Biosystems, Foster City, CA) analyzed using Pedcheck software[13]. The number of complete triads with data on all 4 SNPs was 412 for *ARG1* and 405 for *ARG2*.

Statistical Analysis

Linkage disequilibrium (LD) between pairs of SNPs was measured by computing squared correlation coefficient (r^2) and Lewontin's standardized disequilibrium coefficient (D′)[14, 15].

To analyze associations with individual SNPs, we used a log-linear-based method implemented in SAS version 9.1 for Windows (SAS Institute, Cary, NC) and STATA version 8.0 (Stata Press, College Station, TX) to test for asymmetric distribution of a particular variant allele

among affected offspring compared to Mendelian expectation based on their biologic parents[6]. The method uses genotypes of cases and their parents (triads) stratified into the 15 possible types of triads. Genetic effects produce frequency distortions within parental mating types that permit estimation of genotype relative risks (RR) and 95% confidence intervals (CI) through the log-linear model. A null hypothesis that a SNP is not associated or linked with the outcome is tested by means of the likelihood ratio test. We calculated relative risks for individual SNPs without restricting to a specific genetic model and also under the dominant and recessive genetic models. We also calculated likelihood ratio tests and relative risks for the joint effects of genotype and parent smoking, using the method of Umbach and Weinberg[16].

We analyzed associations with haplotypes, as opposed to single SNPs, using HAPLIN Version 1.0 (http://www.uib.no/smis/gjessing/genetics/software/haplin) running under S-PLUS Version 6.2 for Windows (Seattle, Washington). HAPLIN estimates single- and double-dose effects of haplotypes using maximum likelihood[17]. HAPLIN is a direct extension of the log-linear models for a single diallelic locus[6] although it currently requires complete triad data. The haplotypes of individuals with unknown phase are reconstructed from the triad family information whenever possible, and the remaining are estimated using the expectation-maximization algorithm. The increase in standard error due to the unknown phase is accounted for using a jackknifing procedure. For effects of one specific haplotype the reference group consists of individuals with all remaining haplotypes. The single-dose relative risk then measures the change in risk for an individual carrying exactly one copy of the specific haplotype, whereas the double-dose estimate is the risk for homozygotic individuals relative to the reference group. The rare haplotypes (below 5%) had to be excluded from the analyses because it is difficult to obtain valid relative risk estimates for these haplotypes. Thus, triads including individuals with one or more of these rare haplotypes were excluded, leading to smaller numbers of triads available for the full haplotype analyses than for the single SNP analyses. The HAPLIN analysis includes 360 complete triads for *ARG1* and 310 complete triads for *ARG2*.

To examine whether arginase polymorphisms influenced the degree of atopy, as assessed by the number of positive skin tests out of 24 done, we tested for linkage and association with each of the four SNPs in *ARG1* and *ARG2* using the polytomous logistic method of Kistner and Weinberg[18]. P values were calculated from likelihood ratio tests. For readers more familiar with transmission disequilibrium test (TDT) based methods, we also calculated P values for the individual SNP analyses using FBAT[19]. As expected, P values were very close by the two methods.

RESULTS

Cases had mean age of 9.1 years (standard deviation 2.5 years) and 58% were male. Most had mild (69%) as opposed to moderate or severe asthma (31%). Nearly all cases (98.4%) had used medication for asthma in the past 12 months. Wheezing in the past 12 months was reported by 93% and chronic dry cough was reported by 62.5%. For 71.8% of cases, asthma symptoms had interfered with daily activities or school attendance in the past 12 months. Exacerbation of asthma symptoms by exercise was reported by 72.1% of cases. Among the 331 cases with spirometry data available, FEV_1 was > 80% predicted for 23.5%, 60–80% predicted for 72.3% and < 60% predicted for 4.2%. Atopy to aeroallergens, defined as at least one positive skin test, was present in 93% of cases and 55% of cases had 5 or more positive skin tests. The highest rates of skin test positivity were seen for cockroach (42.6%) and dust mite (71.9%). A smoking parent was present for 52.1% of cases; only 6% of mothers reported smoking during pregnancy.

For the 4 SNPs genotyped for each of *ARG1* and *ARG2*, NCBI SNP ID, the SNP location in the gene, and minor allele frequency are presented in TABLE E1 in the Online Repository. Measures of linkage disequilibrium (D′ and r^2) are shown in TABLE E2 in the Online Repository for *ARG1* and TABLE E3 in the Online Repository for *ARG2*. For *ARG1*, arg1s1 and arg1s2 were highly associated ($r^2 = 0.974$). For *ARG2*, the arg2s1 and arg2s2 SNPs were highly associated ($r^2 = 0.937$).

The frequency distributions of the 15 possible mating types of triads for the polymorphisms of *ARG1* and *ARG2* are shown in TABLE I. The log-linear analysis of individual SNPs in *ARG1* showed no association with asthma (TABLE II). For *ARG2*, carrying 2 copies of minor alleles, for either arg2s1 or the highly associated SNP arg2s2 was associated with a statistically significantly increased risk of asthma (RR = 1.5, 95% CI = 1.1–2.1 for arg2s1, P = 0.018; RR = 1.6, 95% CI = 1.1–2.3 for arg2s2, P = 0.007) (TABLE II). The other two *ARG2* SNPs were not appreciably associated with asthma risk. The corresponding P values from TDT-based analysis were 0.020 for arg2s1 and 0.008 for arg2s2.

Sample sizes are reduced for analyses of haplotypes. We did not observe any significant associations for *ARG1* (TABLE III). For *ARG2*, the haplotype results reflected the single SNP finding in magnitude and direction, although given the reduced sample size the results were not statistically significant. The relative risk was 1.40 (95% CI = 0.84–2.30) for individuals carrying two copies of the haplotype containing the minor alleles for the arg2s1 and arg2s2 with the major alleles at the other two SNPs.

The association between carrying two copies of the minor allele for arg2s1 or arg2s2 was slightly stronger among moderate to severe asthmatics (RR = 2.1, 95% CI = 1.2 – 3.9 for arg2s1, RR = 2.0, 95% CI = 1.1 – 3.6 for arg2s2) than among mild asthmatics (RR = 1.2, 95% CI = 0.8–1.9 for arg2s1, RR = 1.4, 95% CI = 0.9 – 2.2 for arg2s2).

Because parental smoking is a consistent risk factor for childhood asthma, we examined whether the associations differed by whether the case had a smoking parent. The increased risk for carrying two copies of the minor allele for arg2s2 SNP appeared to be limited to cases with smoking parents (RR = 2.39, 95% CI = 1.48–3.86 with smoking parents, RR = 1.06, 95% CI = 0.62–1.81 with nonsmoking parents, P value for likelihood ratio test of gene by environment interaction = 0.025 under recessive model). A similar result was obtained for the very highly associated arg2s1 SNP.

We examined the relationship between individual SNPs of *ARG1* and *ARG2* and the degree of atopy to aeroallergens, assessed by the number of positive skin tests out of a battery of 24 tests. To select the most appropriate genetic model for analyses of these data, we examined the mean number of positive skin tests by number of copies of the variant alleles for the four *ARG1* SNPs (TABLE IV). For all four *ARG1* SNPs, the number of positive tests varied significantly by genotype. The change in number of positive skin tests across the *ARG1* genotypes suggests similar differences in magnitude for going from 0 to 1 copy as for going from 1 to 2, consistent with an additive genetic model. Under the additive model, we observed significant evidence of linkage plus association for all 4 *ARG1* SNPs (TABLE IV). Only one of the four *ARG2* SNPs (arg2s4) showed evidence of linkage and association with the number of positive skin tests (P = 0.027). Due to the limited number of children with two copies of the variant allele (only four) and the sign of the estimate associated with the model, it can be concluded that the significant difference corresponds to an increase in the number of positive skin tests for children with 1 copy as compared to children with no copies of the variant. We calculated odds ratios, under the additive model, for a five unit change in the number of positive skin tests. The magnitude of the associations was similar for the five SNPs (4 in *ARG1* and 1 in *ARG2*) that were statistically significantly associated with number of positive skin tests. P values from

TDT based analyses were virtually identical (range 0.009 to 0.021 for the 4 *ARG1* SNPs and 0.027 for arg2s4).

DISCUSSION

We found genetic variation in *ARG2* to be associated with childhood asthma risk in our Mexican population. Carrying two copies of the minor allele of either of two highly associated SNPs in *ARG2* (arg2s1 or arg2s2) conferred a modest but statistically significantly increased risk to asthma. The modest effect size, with a 40% increase in risk for minor allele homozygotes, is of the magnitude expected for individual SNPs in genes related to asthma[20], a complex disease involving genetic and environmental causes and their interaction. Haplotype results agreed with the single SNP findings, although given the reduced sample size, they were less precise. We also found evidence that *ARG1* SNPs were related to the degree of atopy to aeroallergens, as defined by the number of positive skin tests. All four of the *ARG1* SNPs tested showed evidence of linkage plus association. In addition, one SNP in *ARG2* showed a statistically significant result for atopy to aeroallergens.

Recent work by Zimmermann *et al*. implicates arginase in the etiology of asthma in mouse models and humans[1]. Expression profiling of lung tissue from mice with experimental asthma in response to two distinct allergens revealed markedly increased expression of the two arginase isoforms, *ARG1* and *ARG2*[1]. Total arginase protein activity also increased notably following the induction of asthma in these mouse models. Using *in situ* hybridization for *ARG1* mRNA, Zimmermann *et al.*[1] found localization to macrophages in areas of lung inflammation from the affected mice. Arginase protein by immunohistochemistry also localized to macrophages in areas of peribronchiolar and perivascular inflammation. In contrast, mice without the allergic asthma phenotype had undetectable arginase protein expression. Extending the finding to humans, there was significantly higher arginase protein expression in bronchoalveolar lavage macrophages from asthmatic than nonasthmatic individuals. *ARG1* mRNA was strongly detected in bronchial biopsies of asthmatic patients but virtually undetectable in tissue from normals. In contrast to the murine findings, *ARG1* mRNA was also observed in epithelial cells in the human bronchial biopsy. Localization studies were not done for *ARG2* mRNA or protein[1].

Arginase could impact asthma pathogenesis in several ways. Inhibition of NO generation, due to reduced arginine bioavailability, provides one mechanism[2, 3]. Arginine serves as a common substrate for both nitric oxide synthase (NOS) and arginase. Arginase catalyzes the transformation of arginine to ornithine, thereby reducing arginine availability for NO formation by NOS. Because arginine is a rate-limiting factor for NOS activity in the normal airway, decreased bioavailabity of arginine to NOS can reduce NO synthesis[21]. Deficiency of NO can induce airway hyperresponsiveness, as shown in allergen challenged guinea-pigs[22–24]. With low concentrations of arginine, NOS generates superoxide and NO that interact to form peroxynitrite which leads to airway cell injury[25].

Another potential mechanism for a role of arginase in asthma pathogenesis relates to increasing production of ornithine, a precursor of polyamines (i.e., putrescine, spermidine and spermine) and proline[2, 3]. Polyamines can control cell proliferation and are important in the airway. The polyamine putrescine increased in asthmatic mice along with increased expression of *ARG1* and *ARG2* genes[1]. Circulating levels of putrescine and spermidine were found to be elevated in patients during asthmatic attacks[26]. Proline is a rate limiting substrate for synthesis of collagen, which is deposited in the subepithelium of asthmatic airways in humans[27]. Production of collagens in asthmatic airway cells can influence proliferation and likely contribute to airway remodeling, a key process in asthma[28]. Interestingly, a recent study revealed elevated serum arginase activity and low plasma arginine concentration in human

asthma patients[29]. Taken together, these studies support a role for arginase in asthma pathogenesis via NO-related mechanisms and increased polyamines and prolines, favoring airway remodeling.

We found *ARG1* to be associated with the degree of atopy, but not asthma. *ARG2* was predominantly associated with asthma and to a lesser degree, atopy. Various lines of data support a greater role for ARG1 in allergic response compared with ARG2. ARG1 is localized to macrophages whereas ARG2 is induced in lung myofibroblasts and epithelium as well[30]. An *in vitro* study using murine macrophages, showed that interleukin-4 (IL-4)/interleukin-13 (IL-13) stimulates arginase activity that depletes arginine levels leaving none available for inducible NOS to generate NO[31]. In a study of immunoglobulin E (IgE) sensitized mouse mast cell lines[32], NO inhibited antigen-induced pro-inflammatory cytokine production. If this NO inhibition of antigen-related cytokine release occurs in human macrophages, then decreased NO could lead to increased pro-inflammatory cytokine production following antigen exposure. Although the T helper lymphocytes type 2 (Th2) cytokines IL-4 and IL-13 induce both *ARG1* and *ARG2* in mouse lung[1], induction of *ARG1* mRNA expression by IL-4 is signal transducer and activator of transcription 6 (*STAT6)* dependent, whereas *ARG2* mRNA expression is *STAT6* independent. Mice deficient in *STAT6* do not make IgE[33], which also supports our finding that *ARG1* may contribute to allergic inflammation.

In our data, *ARG2* was associated with both clinical diagnosis of asthma and asthma severity. Both ARG1 and ARG2 are expressed in mouse lung; in human lung, expression of *ARG2* mRNA may be greater than *ARG1*[34]. In bleomycin-induced lung fibrosis, ARG2 is increased earlier and more persistently than ARG1[30]. Similar to the picture in bleomycin-induced fibrosis, in the allergic mouse model, *ARG2* mRNA production occurred earlier after allergen challenge than *ARG1*[1]. ARG2 appears to foster the creation of prolines and polyamines to a greater degree than ARG1[35] and thus might play a greater role in airway remodeling and thus asthma and asthma severity. Of note, *ARG2*, but not *ARG1*, induction by IL-4 is *STAT6* independent as are features of chronic asthma[36]. Given the apparently greater importance of *ARG2* in processes relevant to airway remodeling, and the time course of *ARG2* expression in relation to antigen challenges, *ARG2* may be more important in the development of chronic asthma than *ARG1*, which may be more involved in the acute allergic asthmatic response. Alternatively, given the lack of complete resequencing data, it may be that our coverage of the both *ARG1* and *ARG2* was not sufficient to rule out that both genes are consistently involved in both asthma and atopy.

Our asthma cases were diagnosed by pediatric allergists at a pediatric allergy specialty clinic of a large public hospital. A limitation of the study is that we did not have tests of bronchial hyperreactivity. However, referral to this pediatric allergy clinic is a tertiary referral and thus children had already been seen by a generalist and then a pediatrician over time for recurrent symptoms. Diagnoses were made on clinical grounds according to previous guidelines[8]. Of note, physician diagnosis of asthma has been used as the major endpoint in linkage studies of asthma that have resulted in the positional cloning of asthma genes[37–40]. We had objective data on atopy; skin prick tests revealed the vast majority of these asthmatic children (93%) to be atopic to aeroallergens.

A major limitation of our study was our inability to generate true haplotypes for *ARG1* and *ARG2* because complete resequencing data were not available. However, we felt that it was of interest to examine arginase SNPs taking advantage of public access data with frequency information. In the absence of complete resequencing data, we selected SNPs spaced widely apart from the 5′ to the 3′ end from existing databases. We genotyped four SNPs spread across the locus regions of each gene. In our Mexican population common SNPs were not in the coding region and thus we genotyped SNPs in noncoding regions. Although intronic SNPs may affect

gene expression and function, we have no functional data on these SNPs. A limitation of our study is that there is no functional data available on any SNPs in either gene. A further limitation is that some of our SNPs were highly associated in our Mexican population and thus did not provide independent information. Although arg2s1 and arg2s2 are very highly associated, arg2s1 has been classified as being in exon 1 of the predicted gene *LOC441689* with a product similar to protein kinase C. It is possible that a SNP in this nearby gene is functionally important or that there is a gene-gene interaction with *ARG2*. It is also possible that the LD block including arg2s1 and arg2s2 along with *LOC441689* and other genes, contains a SNP in a regulatory or coding region that might influence gene expression or function. Although *ARG2* lies relative close (2 megabase) to an asthma linkage region on chromosome 14, the r^2 values between SNPs closest to the linkage peak and arg2s1 ranged from 0.05 to 0.13 making it unlikely that our findings were due to linkage equilibrium with SNPs in this region.

We found suggestive evidence of a stronger association between asthma and SNPs in *ARG2* among children from homes where a parent smokes. Environmental tobacco smoke is a stimulus for airway inflammation[41] that might magnify genetic effects that influence this pathway. In addition to effects on inflammatory cells, smoking can decrease endothelial nitric oxide synthase (eNOS) activity in smooth muscle[42] which, if occurring in the airway, would result in decreased bronchodilatory NO. Because arginase activity depletes arginine, leading to decreased substrate for eNOS, there could be a synergistic decrease in NO production. There may also be synergism based on airway remodeling, which both smoking and arginase activity can promote[43]. A limitation of the case-parent triad design in the assessment of gene-environment interaction is that the independence of exposure and genotype must be assumed. However, we have no reason to suspect that the arginase genotype would alter smoking behavior.

Our data suggest associations with between childhood asthma risk and two highly associated SNPs in *ARG2* and the degree of atopy and all four SNPs in *ARG1*. Given the strong biologic rationale for a role of arginases in allergic asthma risk, these findings merit consideration in other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Zimmermann N, King NE, Laporte J, Yang M, Mishra A, Pope SM, et al. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. J Clin Invest 2003;111:1863–74. [PubMed: 12813022]
2. Vercelli D. Arginase: marker, effector, or candidate gene for asthma? J Clin Invest 2003;111:1815–7. [PubMed: 12813015]
3. Meurs H, Maarsingh H, Zaagsma J. Arginase and asthma: novel insights into nitric oxide homeostasis and airway hyperresponsiveness. Trends Pharmacol Sci 2003;24:450–5. [PubMed: 12967769]
4. Hakonarson H, Bjornsdottir US, Halapi E, Palsson S, Adalsteinsdottir E, Gislason D, et al. A major susceptibility gene for asthma maps to chromosome 14q24. Am J Hum Genet 2002;71:483–91. [PubMed: 12119603]

5. Malerba G, Pignatti PF. A review of asthma genetics: gene expression studies and recent candidates. J Appl Genet 2005;46:93–104. [PubMed: 15741670]
6. Weinberg CR, Wilcox AJ, Lie RT. A log-linear approach to case-parent-triad data: assessing effects of disease genes that act either directly or through maternal effects and that may be subject to parental imprinting. Am J Hum Genet 1998;62:969–78. [PubMed: 9529360]
7. Wilcox AJ, Weinberg CR, Lie RT. Distinguishing the effects of maternal and offspring genes through studies of "case-parent triads". Am J Epidemiol 1998;148:893–901. [PubMed: 9801020]
8. BTS/SIGN. British guideline on the management of asthma. Thorax 2003;58 (Suppl 1):i1–94. [PubMed: 12653493]
9. NHLBI. Pocket Guide for Asthma Management and Prevention: Global Initiative for Asthma. NIH Publication: National Institutes of Health, 1998. Bethesda, MD 20892
10. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med 1995;152:1107–36. [PubMed: 7663792]
11. Perez-Padilla R, Regalado-Pineda J, Rojas M, Catalan M, Mendoza L, Rojas R, et al. Spirometric function in children of Mexico City compared to Mexican-American children. Pediatr Pulmonol 2003;35:177–83. [PubMed: 12567385]
12. Aas K, Belin L. Standardization of diagnostic work in allergy. Int Arch Allergy Appl Immunol 1973;45:57–60. [PubMed: 4580380]
13. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259–66. [PubMed: 9634505]
14. Devlin B, Risch N. A comparison of linkage disequilibrium measures for fine-scale mapping. Genomics 1995;29:311–22. [PubMed: 8666377]
15. Lewontin RC. On measures of gametic disequilibrium. Genetics 1988;120:849–52. [PubMed: 3224810]
16. Umbach DM, Weinberg CR. The use of case-parent triads to study joint effects of genotype and exposure. Am J Hum Genet 2000;66:251–61. [PubMed: 10631155]
17. Gjessing HK, Lie RT. Case-Parent Triad data: Estimating single- and double-dose effects of fetal and maternal disease gene haplotypes. Annals of Human Genetics 2005; in press. available from Blackwell on Online Early: http://www.blackwell-synergy.com/doi/pdf/10.1111/j.1529–8817.2005.00218.x
18. Kistner EO, Weinberg CR. Method for using complete and incomplete trios to identify genes related to a quantitative trait. Genet Epidemiol 2004;27:33–42. [PubMed: 15185401]
19. Horvath S, Xu X, Lake SL, Silverman EK, Weiss ST, Laird NM. Family-based tests for associating haplotypes with general phenotype data: application to asthma genetics. Genet Epidemiol 2004;26:61–9. [PubMed: 14691957]
20. Kormann MS, Carr D, Klopp N, Illig T, Leupold W, Fritzsch C, et al. G-Protein-coupled Receptor Polymorphisms Are Associated with Asthma in a Large German Population. Am J Respir Crit Care Med 2005;171:1358–62. [PubMed: 15764725]
21. de Boer J, Duyvendak M, Schuurman FE, Pouw FMH, Zaagsma J, Meurs H. Role of L–arginine in the deficiency of nitric oxide and airway hyperreactivity after the allergen-induced early asthmatic reaction in guinea-pigs. Br J Pharmacol 1999;128:1114–20. [PubMed: 10556950]
22. de Boer J, Meurs H, Coers W, Koopal M, Bottone AE, Visser AC, et al. Deficiency of nitric oxide in allergen-induced airway hyperreactivity to contractile agonists after the early asthmatic reaction: an ex vivo study. Br J Pharmacol 1996;119:1109–16. [PubMed: 8937712]
23. Mehta S, Drazen JM, Lilly CM. Endogenous nitric oxide and allergic bronchial hyperresponsiveness in guinea pigs. Am J Physiol Lung Cell Mol Physiol 1997;273:L656–62.
24. Miura M, Yamauchi H, Ichinose M, Ohuchi Y, Kageyama N, Tomaki M, et al. Impairment of Neural Nitric Oxide-mediated Relaxation after Antigen Exposure in Guinea Pig Airways in vitro. Am. J. Respir. Crit. Care Med 1997;156:217–22.
25. Xia Y, Dawson VL, Dawson TM, Snyder SH, Zweier JL. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proc Natl Acad Sci U S A 1996;93:6770–4. [PubMed: 8692893]

26. Kurosawa M, Shimizu Y, Tsukagoshi H, Ueki M. Elevated levels of peripheral-blood, naturally occurring aliphatic polyamines in bronchial asthmatic patients with active symptoms. Allergy 1992;47:638–43. [PubMed: 1285570]
27. Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. Clin Exp Allergy 1997;27:363–71. [PubMed: 9146928]
28. Johnson PRA, Burgess JK, Underwood PA, Au W, Poniris MH, Tamm M, et al. Extracellular matrix proteins modulate asthmatic airway smooth muscle cell proliferation via an autocrine mechanism. Journal of Allergy and Clinical Immunology 2004;113:690–6. [PubMed: 15100675]
29. Morris CR, Poljakovic M, Lavrisha L, Machado L, Kuypers FA, Morris SM Jr. Decreased Arginine Bioavailability and Increased Serum Arginase Activity in Asthma. Am. J. Respir. Crit. Care Med 2004;170:148–53.
30. Endo M, Oyadomari S, Terasaki Y, Takeya M, Suga M, Mori M, et al. Induction of arginase I and II in bleomycin-induced fibrosis of mouse lung. Am J Physiol Lung Cell Mol Physiol 2003;285:L313–21. [PubMed: 12679322]
31. Rutschman R, Lang R, Hesse M, Ihle JN, Wynn TA, Murray PJ. Cutting Edge: Stat6-Dependent Substrate Depletion Regulates Nitric Oxide Production. J Immunol 2001;166:2173–7. [PubMed: 11160269]
32. Davis BJ, Flanagan BF, Gilfillan AM, Metcalfe DD, Coleman JW. Nitric oxide inhibits IgE-dependent cytokine production and Fos and Jun activation in mast cells. J Immunol 2004;173:6914–20. [PubMed: 15557187]
33. Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 1996;380:630–3. [PubMed: 8602264]
34. Gotoh T, Araki M, Mori M. Chromosomal Localization of the Human Arginase II Gene and Tissue Distribution of Its mRNA. Biochemical and Biophysical Research Communications 1997;233:487–91. [PubMed: 9144563]
35. Cederbaum SD, Yu H, Grody WW, Kern RM, Yoo P, Iyer RK. Arginases I and II: do their functions overlap? Mol Genet Metab 2004;81 (Suppl 1):S38–44. [PubMed: 15050972]
36. Zimmermann N, Mishra A, King NE, Fulkerson PC, Doepker MP, Nikolaidis NM, et al. Transcript Signatures in Experimental Asthma: Identification of STAT6-Dependent and -Independent Pathways. J Immunol 2004;172:1815–24. [PubMed: 14734765]
37. Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 2002;418:426–30. [PubMed: 12110844]
38. Laitinen T, Polvi A, Rydman P, Vendelin J, Pulkkinen V, Salmikangas P, et al. Characterization of a Common Susceptibility Locus for Asthma-Related Traits. Science 2004;304:300–4. [PubMed: 15073379]
39. Allen M, Heinzmann A, Noguchi E, Abecasis G, Broxholme J, Ponting CP, et al. Positional cloning of a novel gene influencing asthma from Chromosome 2q14. Nat Genet 2003;35:258–63. [PubMed: 14566338]
40. Zhang Y, Leaves NI, Anderson GG, Ponting CP, Broxholme J, Holt R, et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. Nat Genet 2003;34:181–6. [PubMed: 12754510]
41. Floreani AA, Rennard SI. The role of cigarette smoke in the pathogenesis of asthma and as a trigger for acute symptoms. Curr Opin Pulm Med 1999;5:38–46. [PubMed: 10813248]
42. Hutchison SJ, Sievers RE, Zhu B-Q, Sun Y-P, Stewart DJ, Parmley WW, et al. Secondhand Tobacco Smoke Impairs Rabbit Pulmonary Artery Endothelium-Dependent Relaxation. Chest 2001;120:2004–12. [PubMed: 11742935]
43. Smith KR, Pinkerton KE, Watanabe T, Pedersen TL, Ma SJ, Hammock BD. Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. Proc Natl Acad Sci U S A 2005;102:2186–91. [PubMed: 15684051]

TABLE I

Distribution of case-parent triad genotypes for *ARG1* 4 SNPs and *ARG2* 4 SNPs

Mother-Father-Child*	Triad Counts (*ARG1*)				Triad Counts (*ARG2*)			
	s1	s2	s3	s4	s1	s2	s3	s4
000	27	28	30	70	65	71	79	206
010	29	28	28	45	34	39	39	35
011	21	22	29	43	28	31	41	36
021	21	20	31	25	19	15	20	12
100	32	33	38	29	37	41	34	42
101	29	29	29	36	36	36	33	34
110	24	24	30	11	23	22	21	5
111	49	48	50	47	42	40	52	15
112	32	34	27	21	27	28	18	3
121	26	26	19	15	12	10	8	2
122	29	28	22	9	18	17	16	0
201	30	30	20	29	27	23	21	13
211	22	23	20	15	7	6	9	1
212	20	20	23	8	20	18	7	1
222	21	19	16	9	10	8	7	0
Total	412	412	412	412	405	405	405	405

* Number of copies of minor allele in mother-father-child

TABLE II

Childhood asthma risk in relation to *ARG1* and *ARG2* SNPs.

ARG1				*ARG2*			
SNPs	**Model**	**Genotype**	**RR (95% CI)**	**SNPs**	**Model**	**Genotype**	**RR (95% CI)**
s1				s1			
	Unrestricted	AA	1.0		Unrestricted	GG	1.0
		AG	0.9 (0.7–1.2)			GC	0.9 (0.7–1.2)
		GG	1.1 (0.7–1.6)			CC	1.4 (0.9–2.0)
	Dominant	AG + GG	0.9 (0.7–1.2)		Dominant	GC + CC	0.9 (0.7–1.2)
	Recessive	AA + AG	1.0		Recessive	GG + GC	1.0
		GG	1.2 (0.9–1.5)			CC	1.5 (1.1–2.1)*
s2				s2			
	Unrestricted	TT	1.0		Unrestricted	TT	1.0
		TA	0.9 (0.7–1.2)			TC	0.8 (0.6–1.1)
		AA	1.1 (0.8–1.6)			CC	1.4 (0.9–2.1)
	Dominant	TA + AA	1.0 (0.7–1.3)		Dominant	TC + CC	0.9 (0.7–1.2)
	Recessive	TT + TA	1.0		Recessive	TT + TC	1.0
		AA	1.2 (0.9–1.6)			CC	1.6 (1.1–2.3)†
s3				s3			
	Unrestricted	CC	1.0		Unrestricted	GG	1.0
		CT	0.9 (0.7–1.1)			GA	1.1 (0.8–1.4)
		TT	1.0 (0.7–1.4)			AA	1.0 (0.6–1.5)
	Dominant	CT + TT	0.9 (0.7–1.1)		Dominant	GA + AA	1.0 (0.8–1.4)
	Recessive	CC + CT	1.0		Recessive	GG + GA	1.0
		TT	1.1 (0.8–1.5)			AA	0.9 (0.6–1.4)
s4				s4			
	Unrestricted	TT	1.0		Unrestricted	TT	1.0
		TC	1.3 (1.0–1.7)			TC	1.0 (0.7–1.3)
		CC	1.0 (0.6–1.6)			CC	0.5 (0.2–1.4)
	Dominant	TC + CC	1.2 (0.9–1.6)		Dominant	TC + CC	0.9 (0.7–1.3)
	Recessive	TT + TC	1.0		Recessive	TT + TC	1.0
		CC	0.8 (0.6–1.2)			CC	0.5 (0.2–1.4)

CI, confidence interval

RR, relative risk

* P = 0.018

† P = 0.007

TABLE III

Haplotype relative risks for childhood asthma in relation to *ARG1* and *ARG2*.

Haplotype*	Frequency (95% CI)	Relative Risk (95% CI)	
		Single Copy	Double Copy
ARG1			
ATTT	0.46 (0.43–0.50)	0.87 (0.63–1.20)	0.85 (0.54–1.30)
GACC	0.29 (0.26–0.33)	1.00 (0.73–1.40)	1.00 (0.61–1.80)
GACT	0.20 (0.17–0.23)	1.10 (0.82–1.50)	0.60 (0.27–1.30)
ATCC	0.05 (0.04–0.07)	1.00 (0.61–1.60)	1.90 (0.41–8.80)
ARG2			
GTAT	0.37 (0.34–0.41)	1.10 (0.81–1.60)	1.10 (0.65–1.80)
CCGT	0.31 (0.28–0.35)	1.00 (0.76–1.50)	1.40 (0.84–2.30)
GTGT	0.17 (0.15–0.21)	0.82 (0.58–1.20)	1.00 (0.48–2.20)
GTGC	0.14 (0.12–0.17)	1.00 (0.72–1.50)	0.34 (0.08–1.50)

CI, confidence interval

* Haplotypes formed by arg1s1, arg1s2, arg1s3, arg1s4 in order for *ARG1*; by arg2s1, arg2s2, arg2s3, arg2s4 in order for *ARG2*.

TABLE IV

Relation between number of positive skin tests and arginase genotypes

SNP	# copies of minor allele	# of cases	# positive skin tests (mean)	OR (95% CI)*	P value for linkage and association†
ARG1					
s1	0	109	4.96	1.00	
	1	194	5.77	1.34(1.05–1.71)	
	2	97	6.72	1.80(1.10–2.92)	0.017
s2	0	110	4.96	1.00	
	1	194	5.77	1.35(1.05–1.72)	
	2	96	6.75	1.81(1.11–2.95)	0.016
s3	0	121	6.47	1.00	
	1	194	5.72	0.75(0.59–0.95)	
	2	85	4.95	0.56(0.34–0.91)	0.018
s4	0	151	5.32	1.00	
	1	203	5.91	1.42(1.09–1.85)	
	2	46	6.76	2.02(1.20–3.41)	0.007
ARG2					
s1	0	153	6.10	1.00	
	1	166	5.42	0.89(0.69–1.14)	
	2	73	5.86	0.78(0.47–1.29)	0.343
s2	0	167	6.07	1.00	
	1	156	5.46	0.86(0.67–1.11)	
	2	69	5.72	0.75(0.45–1.23)	0.245
s3	0	171	5.63	1.00	
	1	176	6.16	0.94(0.74–1.20)	
	2	45	4.73	0.88(0.54–1.43)	0.612
s4	0	277	5.51	1.00	
	1	111	6.48	1.48(1.04–2.10)	
	2	4	4.00	2.18(1.08–4.41)	0.027

CI, confidence interval

*The odds ratio (OR) is interpreted as the change in odds of inheriting one or two copies of the variant allele per 5 unit change in the number of positive skin tests.

†P values and odds ratios are based on the additive genetic model