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Genetic Variation in S-nitrosoglutathione Reductase (*GSNOR*) and Childhood Asthma

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Abstract

Background— S-nitrosothiols are potent endogenous bronchodilators depleted in asthmatic airway lining fluid. S-nitrosoglutathione reductase (GSNOR, also known as ADH5 or FDH) catalyzes the metabolism of S-nitrosoglutathione (GSNO) and controls intracellular levels of S-nitrosothiols. *GSNOR* knockout mice have increased lung S-nitrosothiols and are therefore protected from airway hyperresponsiveness after methacholine or allergen challenge.

Objective— To investigate whether genetic variation in *GSNOR* is associated with childhood asthma and atopy.

Methods— We genotyped 5 tagging and two additional single nucleotide polymorphisms (SNPs) in *GSNOR*, in 532 nuclear families consisting of asthmatic children aged 4 to 17 years and both parents in Mexico City. Atopy was determined by skin prick tests.

Results— Carrying one or two copies of the minor allele of SNP rs1154404 was associated with decreased risk of asthma [relative risk (RR) = 0.77, 95% confidence interval (CI), 0.61–0.97, P = 0.028 for one copy; RR = 0.66, 95% CI, 0.44–0.99, P = 0.046 for two copies]. Homozygosity for the minor allele of SNP rs28730619 was associated with increased risk of asthma (RR = 1.60, 95% CI, 1.13–2.26, P = 0.0077). Haplotype analyses supported the single SNP findings. *GSNOR* SNPs were not associated with the degree of atopy.

Conclusion— This is the first study of genetic polymorphisms in *GSNOR* and asthma. These data suggest that genetic variation in *GSNOR* may play a role in asthma susceptibility.

Clinical implications— The association of *GSNOR* polymorphisms with asthma suggests a potential therapeutic target.

Capsule Summary— Findings from this study suggest that genetic variation in S-nitrosoglutathione reductase (GSNOR) may play a role in asthma susceptibility.

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Keywords

alcohol dehydrogenase 5 (ADH5); allergy; asthma; formaldehyde dehydrogenase (FDH); genetic predisposition to disease; nitric oxide (NO); single nucleotide polymorphism (SNP); S-nitrosoglutathione (GSNO); S-nitrosoglutathione reductase (GSNOR); S-nitrosothiol (SNO)

INTRODUCTION

Asthma is a complex disease characterized by inflammation and hyperresponsiveness of the airways. Nitric oxide (NO) signaling pathways have been implicated in the regulation of airway hyperresponsiveness in asthma.[1, 2] NO levels are higher in the exhaled air of asthmatics than unaffected individuals;[3] cytokine induced over-expression of NO synthase in the airways likely contributes to the increase.[4, 5] The biological function of NO is conveyed mainly through S-nitrosylation of cysteine residues of proteins to form more stable S-nitrosothiols.[6]

S-nitrosothiols are potent endogenous bronchodilators that are depleted from the airway lining fluid in human asthmatics,[7, 8] suggesting that they may play a protective role against airway hyperresponsiveness. The most common S-nitrosothiol in the airway is S-nitrosoglutathione (GSNO).[7] S-nitrosoglutathione reductase (GSNOR) is the major enzyme that catalyzes GSNO metabolism and controls intracellular levels of S-nitrosothiols.[9] Recent evidence demonstrates that GSNOR activity is increased in asthmatic lungs, resulting in diminished S-nitrosothiols and leading, in turn, to airway hyperresponsiveness.[2] *GSNOR* gene knockout mice have increased lung S-nitrosothiols and are protected from airway hyperresponsiveness after methacholine or allergen challenge, suggesting that GSNOR is a crucial modulator of airway tone.[2]

Due to the accumulating evidence for a role of GSNOR in asthma pathogenesis,[2, 10, 11] we used the case-parent triad design to investigate associations of *GSNOR* polymorphisms and haplotypes with childhood asthma and atopy in asthmatic children from Mexico City. We are aware of no published data on genetic variation in *GSNOR* and asthma risk.

METHODS

Study design and subject enrollment

We used the case-parent triad design.[12, 13] The study population included 532 case-parent triads with adequate DNA samples for genotyping of at least one SNP for the *GSNOR* genes. The 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 México, Federico Gómez). Children and parents provided blood samples as sources of DNA. A parent, nearly always the mother, completed a questionnaire on the child’s symptoms and risk factors for asthma including current parental smoking, parental smoking during the first two years of the child’s life, maternal smoking during pregnancy, and residential history.

We obtained measurements of ambient ozone from the Mexican government’s air monitoring stations. Ozone levels were measured via UV photometry (analyzer-model 400, API). The residence of each child who participated in this study was located using a map and the closest monitoring station was assigned to that residence.[14] The ozone exposure data were collected for the year prior to the time of entry into the study. The parameter we used was the annual average of the daily maximum 8 hour averages. We dichotomized this variable at the median of 67 ppb for stratified analyses.

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The protocol was reviewed and approved by the Institute Review Boards of the Mexican National Institute of Public Health, the Hospital Infantil de México, Federico Gómez, and the U.S. National Institute of Environmental Health Sciences. Parents provided the written informed consent for the child's participation. Children also gave their informed assent.

Clinical evaluation

The diagnosis of asthma was based on clinical symptoms and response to treatment by a pediatric allergist.[15] The severity of asthma was rated by a pediatric allergist for 508 cases according to symptoms in the Global Initiative on Asthma schema as mild (intermittent or persistent), moderate or severe.[16] At a later date, for research purposes, pulmonary function was measured using the EasyOne spirometer (ndd Medical Technologies, Andover, MA, USA) for 401 cases according to ATS specifications.[17] 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).[18] Children were asked to hold asthma medications on the morning of the test.

Atopy was determined using skin prick tests. The following battery of 24 aeroallergens (IPI ASAC, Mexico) common in Mexico City was used: *Aspergillus fumigatus*, *Alternaria*, *Mucor*, *Blattella germanica*, *Periplaneta americana*, *Penicillium*, cat, dog, horse, *Dermatophagoides (pteronyssinus and farina)*, *Ambrosia*, *Artemisa ludoviciana*, *Cynodon dactylon*, *Chenopodium album*, *Quercus robur*, *Fraxinus*, *Helianthus annus*, *Ligustrum vulgare*, *Lolium perenne*, *Plantago lanceolata*, *Rumex crispus*, *Schinus molle*, *Salsola* and *Phleum pratense*. Histamine was used as a positive control and glycerin as a 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 the skin prick test recommended by Aas and Belin.[19] Skin test data on all 24 aeroallergens were available on 483 cases.

SNP selection

We selected tagging SNPs based on resequencing data in 22 Mexican-Americans with four grandparents born in Mexico in the NIEHS Environmental Genome Project (http://egp.gs.washington.edu). We identified the common (frequency > 5%) haplotypes using PHASE[20] and then used ldSelect[21] to identify tagging SNPs. In ldSelect, a bin is formed by a group of SNPs where a given SNP (the tagging SNP) is in high linkage disequilibrium ($r^2 > 0.8$ in our analysis) with the maximum number of other SNPs. There is a trade-off between SNP coverage and power in association studies.[22] When nothing is known about function, selecting SNPs in bins that contain at least one other SNP improves efficiency.[22] We aimed to select a sufficient number of SNPs to cover multiple SNP bins and common haplotypes in the gene. There were 5 SNPs with minor allele frequencies over 10%, rs1154401, rs1154404, rs1154410, rs1154412 and rs28730619 (Table E1 in the Online Repository), that would distinguish all five common haplotypes based on the resequencing data (Table E2 in the Online Repository). Thus, these are the five SNPs of a priori interest. We also genotyped SNPs, rs1154411 and rs1154409, both in repeat regions and in single SNP bins, to cover all bins. However, these two additional SNPs did not provide additional information on the relationship between common haplotypes and asthma. To the best of our knowledge, no functional GSNOR SNPs have been identified in the literature.

Genotyping

DNA was extracted from peripheral blood lymphocyte using Gentra Puregene kits (Gentra System, Minneapolis, MN). Genotypes for the rs1154404, rs1154410 and rs1154412 were obtained using TaqMan SNP Genotyping Assay.[23] Primers and probes were purchased from Assay-on-Demand (Applied Biosystems, Foster City, CA). All PCR amplifications were

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performed using 5′ exonuclease assay on GeneAmp PCR Systems 9700 (Applied Biosystems). The fluorescence of PCR products was detected using ABI Prism 7900HT sequence detection system. The rs1154401 and rs28730619 SNPs were genotyped using MGB Eclipse Genotyping Assay.[24] Primers and probes were purchased from MGB Eclipse by Design (Epoch Biosciences, Bothell, WA). Because rs1154409 and rs1154411 are located in long repeat regions, it was not possible to design either TaqMan or MGB Eclipse assays for them and thus they were genotyped using the MALDI-TOF MS hME genotyping assay (http://www.bioserve.com/services/genotyping/maldiTofMsHme.cfm) by BioServe Biotechnologies, Ltd. (Laurel, MD). All genotyping assays were done by a researcher who was blinded to parent or child status of samples. Sixteen quality control samples were plated per 384-well plate along with 24 control samples with known genotype. An additional six blind replicate samples were included in the analyses. The quality controls and the blind replicates were 100% concordant for all genotyping methods. All genotyping data reflected at least 95% plate assay efficiency.

Non-parentage was ascertained with a set of short-tandem repeats (AmpFLSTR Profiler Plus; Applied Biosystems) analyzed using Pedcheck software (University of Pittsburgh, Pittsburgh, PA).[25] A total of 532 families had genotyping data for at least one of the 5 tagging SNPs plus the two additional SNPs. Five hundred and thirteen families had genotyping data for all 5 tagging SNPs; with the two additional SNPs, the number with data on all 7 is reduced to 467.

Statistical analysis

We used a log-linear likelihood approach to analyze associations between asthma and individual SNPs.[12] The log-linear likelihood-ratio test is a powerful and more flexible generalization of the transmission disequilibrium test (TDT) and has the advantage of providing estimates of the magnitude of associations rather than simply tests of significance.[12] Similar to TDT based methods for the analysis of case-parent data, such as the family based association test (FBAT),[26] the log-linear model tests the same null hypothesis of no within-family relationship between variant and the disease and achieves robustness against genetic population structure through stratification on the possible parental mating types.[12, 27] The log-linear method thus gives comparable P values to TDT-based methods such as FBAT (see footnotes to Table III and IV for FBAT P values). We calculated relative risks for individual SNPs without restricting to a specific genetic model. The log-linear models of case-parent data are inherently immune to confounding by demographic, lifestyle factors such as parental smoking, or environmental exposures. However, we examined effect modification by gender, asthma severity, parental smoking, and level of ozone exposure. We calculated tests of interactions for the joint effects of genotype and above factors using the method of Umbach and Weinberg.[28] All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) and STATA version 8.0 (Stata Press, College Station, TX).

To evaluate whether *GSNOR* polymorphisms influenced the degree of atopy, as assessed by the number of positive skin tests out of 24 performed, we used the polytomous logistic method of Kistner and Weinberg to estimate the linkage and association between *GSNOR* polymorphisms and atopy.[29] P values were calculated from likelihood ratio tests. We also used this method to analyze the relationship between *GSNOR* SNPs and lung function, as assessed by percent predicted FEV_1.

We used HAPLIN version 2.0 (http://www.uib.no/smis/gjessing/genetics/software/haplin) to analyze associations between asthma and *GSNOR* haplotypes. HAPLIN is an extension of the log-linear model from a single locus to loci with multiple haplotypes with unknown phase.[30] The haplotypes of individuals with unknown phase are constructed from the family information whenever possible, and the remaining haplotypes are estimated by using the expectation-maximization algorithm.[30] HAPLIN estimates single- and double-dose effects of

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haplotypes rather than simply tests of significance using maximum likelihood.[30] We set a threshold of 1% for haplotype frequency leaving 519 families in the HAPLIN analyses for the 5 tagging SNPs. We also performed the extended haplotype analysis including the 5 tagging SNPs and the two additional SNPs, rs1154409 and rs1154411, using HAPLIN.

We present the P values computed using the above methods. To address the potential issue of multiple comparisons, we calculated the false discovery rate for each P value of less than 0.05 using the method of Storey.[31] The false discovery rate is the expected proportion of false positives incurred when a particular test is called significant.[31] However, it should be noted that these corrections will be overly conservative when applied equally to all SNPs. In addition, the false discovery rate does not take into account the correlation between SNPs in a gene. Our calculation of the false discovery rate is based on the 5 tagging SNPs of a priori interest.

RESULTS

Characteristics of the asthmatic children with genotyping data are presented in Table I. The mean age of cases was 9.0 years (range 4–17 years). Most had mild (71.7%) as opposed to moderate or severe asthma (28.3%). Nearly all cases (98.1%) had used medication for asthma in the past 12 months. Wheezing in the past 12 months was reported by 90.3% of the cases and chronic dry cough was reported by 65.0%. For 73.5% of the cases, asthma symptoms had interfered with daily activities or school attendance in the past 12 months. Among cases with spirometry data, the mean FEV_1 percent predicted was 96.8 (SD = 19.7). Atopy was present in 91.7% of cases. The highest rates of skin test positivity were seen for dust mite (70.8%) and cockroach (42.2%). Only 5.0% of mothers reported smoking during pregnancy, but 51.2% had a parent who currently smoked.

The frequency distributions for all mating types are presented in Table II for the 5 tagging *GSNOR* SNPs and in Table E3 in the Online Repository for the two additional SNPs, rs1154409 and rs1154411. The minor allele frequency in parents was 0.19 for rs1154401, 0.31 for rs1154404, 0.09 for rs1154410, 0.13 for rs1154412, 0.45 for rs28730619, 0.05 for rs1154409, and 0.10 for rs1154411. All SNPs except rs1154411 were in Hardy-Weinberg equilibrium ($P > 0.05$) in parents. The pairwise linkage disequilibrium coefficients (r^2) between *GSNOR* SNPs, calculated using FBAT,[26] are shown in Table E4 in the Online Repository. There was moderate linkage disequilibrium between the rs1154401 and rs1154412 SNPs ($r^2 = 0.60$), the rs1154410 and rs1154411 SNPs ($r^2 = 0.58$), and the rs1154401 and rs1154410 SNPs ($r^2 = 0.41$); all other pairwise comparisons gave lower values of r^2.

For *GSNOR*, carrying one or two copies of the minor A allele of the rs1154404 SNP was statistically significantly associated with decreased asthma risk [relative risk (RR) = 0.77, 95% confidence interval (CI), 0.61–0.97, P = 0.028, false discovery rate = 0.06 for one copy; RR = 0.66, 95% CI = 0.44–0.99, P = 0.046, false discovery rate = 0.07 for two copies] relative to homozygotes for the major T allele (Table III). Correspondingly, risk of asthma increased with increasing copies of the major T allele of rs1154404. Carrying two copies of the minor G allele of the rs28730619 SNP was statistically significantly associated with increased asthma risk (RR = 1.60, 95% CI, 1.13–2.26, P = 0.0077, false discovery rate = 0.03). Results for the 5 tagging SNPs did not differ appreciably by gender, asthma severity (mild versus moderate to severe), parental smoking status, or ozone level (data not shown). We did not observe associations between the *GSNOR* SNPs and percent predicted FEV_1 (data not shown).

Haplotype analyses results reflected the single SNP findings in magnitude and direction (Table IV). Carrying two copies of the ht1 (GTCGG) haplotype, the one containing the major T allele of rs1154404 and the minor G allele of rs28730619, exhibited an increased risk of asthma (RR = 1.57, 95% CI, 1.09–2.30, P = 0.017, false discovery rate = 0.02).

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The two additional SNPs genotyped, rs1154409 and rs1154411, that do not contribute to haplotype enumeration, were not associated with asthma (Table E5 in the Online Repository). The haplotype analysis with all 7 SNPs gave similar results to the analysis with the 5 tagging SNPs (Table E6 in the Online Repository).

We examined the association between individual *GSNOR* SNPs and the degree of atopy to aeroallergens, assessed by the number of positive skin tests out of a battery of 24 tests. No significant associations were detected with the number of positive skin tests for the 5 *GSNOR* SNPs (data not shown).

DISCUSSION

Based on accumulating experimental evidence,[2, 10, 11] we examined *GSNOR* as a potential asthma candidate gene. Carrying one or two copies of the minor A allele of the rs1154404 SNP decreased the risk of childhood asthma. This finding translates to an increased risk of asthma among homozygotes for the major T allele of this SNP. For the rs28730619 SNP, homozygotes for the minor G allele also had an increased risk of developing asthma. Results of haplotype analysis agreed with the single SNP findings; the most common haplotype, the one containing the major T allele of rs1154404 and the minor G allele of rs28730619, increased the risk of asthma.

Nitric oxide (NO) plays dual roles in asthma pathogenesis, contributing to airway inflammation through the formation of toxic reactive nitrogen species,[32] or, in contrast, relaxing bronchial smooth muscle and leading to bronchodilation through the formation of S-nitrosothiols.[7, 33] In S-nitrosylation, which occurs largely in proteins, NO covalently modifies cysteine thiols to form S-nitrosothiols. These compounds, in particular S-nitrosoglutathione (GSNO) are the reservoir of NO bioactivity.[7] S-nitrosothiols are potent endogenous bronchodilators that regulate bronchial smooth muscle tone and protect from airway hyperresponsiveness in human asthma.[8] The homeostasis of S-nitrosothiols is modulated by GSNO reductase (GSNOR), a very specific enzyme for GSNO metabolism.[9]

GSNOR, originally known as formaldehyde dehydrogenase (*FDH*), was recently identified by Liu *et al* as a metabolism enzyme for GSNO.[9] The official gene name of *GSNOR* is alcohol dehydrogenase 5 (*ADH5*) which is located in a gene cluster with another five alcohol dehydrogenase genes on chromosome 4. However, *GSNOR* is unique in that its promoter is GC rich and does not have a TATA box.[34] Furthermore, GSNOR differs in catalytic profile from all other alcohol dehydrogenases. For example, it appears to have no activity for ethanol oxidation and its best known substrate is GSNO.[9]

Recent work by Que *et al* with mouse models implicates GSNOR in the etiology of asthma.[2] GSNOR is expressed in multiple cell types in the lung including airway epithelial cells and infiltrating leukocytes.[2] After exposure to ovalbumin, GSNOR activity increased in airway lining fluid, perhaps reflecting its release from injured epithelial or inflammatory cells.[2] After ovalbumin challenge, elevated activity of GSNOR catalyzes the increased breakdown of endogenous S-nitrosothiols which thus become depleted in the human asthmatic airway lining fluid.[2, 7, 8] Decreased concentration of S-nitrosothiols in the airways contributes to bronchoconstriction. *GSNOR* knockout mice do not develop bronchoconstriction after administration of methacholine, a cholinergic agonist.[2] When GSNOR is absent, SNO levels remain elevated, contributing to decreased airway hyperresponsiveness in the knockout mice.[2] Notably, in face of ovalbumin challenge, they develop airway inflammation but not airway hyperresponsiveness.[2] Furthermore, tracheal rings from *GSNOR* knockout mice repeatedly treated with isoproterenol, a β2 adrenergic agonist and airway dilator, maintained their responsiveness whereas those from wild type mice demonstrated a decrease in responsiveness

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after treatment.[2] SNO homeostasis, modulated by GSNOR, regulates both basal airway tone and airway hyperresponsiveness to bronchoconstrictor and allergen challenges.[2] Accelerated S-nitrosothiol turnover catalyzed by GSNOR is not only relevant to rodent airway hyperresponsiveness[2, 35] but also to increased bronchial smooth muscle tone in human asthma.[8, 11, 33]

The two SNPs that we found to be associated with asthma (rs28730619 and rs1154404) are in moderate linkage disequilibrium ($r^2 = 0.35$) with each other. Homozygotes for the most common *GSNOR* haplotype (GTCGG), which contained the major T allele of rs1154404 and the minor G allele of rs28730619, were at increased asthma risk. This result was consistent with the single SNP findings but did not clearly distinguish the relative importance of these two SNPs. These SNPs have not been studied for functional significance and there is no clear basis to predict direct effects on GSNOR production and activity. Given that neither SNP is clearly functional, it is reasonable to speculate that there may be linkage disequilibrium with a functional SNP in this gene or outside. Notably, the tagging SNP rs1154404, located in intron 1, is in virtually complete linkage disequilibrium ($r^2 = 0.99$) with two adjacent promoter SNPs, rs2602899 and rs2851301, in a potential nuclear factor kappa B (NF-κB) binding site.[36,37] Although speculative, carrying the minor allele for these two promoter SNPs may result in the loss of the potential NF-κB binding site and therefore could reduce GSNOR production, leading to increased levels of S-nitrosothiols and thus protection from airway hyperresponsiveness. In theory, this schema could explain the association of rs1154404 with decreased risk of childhood asthma. We did not genotype these two promoter SNPs directly for several reasons; they are next to each other which will impact the genotyping accuracy, they are not tagging SNPs based on linkage disequilibrium criteria, and genotyping these two SNPs will not give additional information because they are essentially in complete linkage disequilibrium with our tagging SNP, rs1154404.

Resequencing data from NIEHS Environmental Genome Project indicates a distinct polymorphic pattern of the GSNOR gene in Asians compared with Hispanics, Europeans, and especially Africans. This difference is consistent with the hypothesis of an African origin for humans. The Asian sample only has three SNPs with minor allele frequencies higher than 10%. One of these three common SNPs is rs28730619, which was associated with asthma in our Mexican population. Therefore, in an Asian population, one might be able to test the genetic effect of rs28730619 in the absence of common polymorphisms found in other populations.

Despite the association with asthma, it is not surprising that we did not find a relation between the percent predicted FEV_1 and SNPs in *GSNOR*. FEV_1 measured on a routine clinic visit is likely to be normal in most asthmatic children.[38] FEV_1 in children reflects a complex mix of factors in the growth and development of the child, of which asthma status is only one factor. The likelihood that asthma candidate SNPs would have a major association with FEV_1 measured at a single point in time in a child would seem to be low.

Our study has several strengths. Our tagging SNPs were selected using sound bioinformatics tools based on resequencing data in Mexican-Americans with four grandparents born in Mexico who could be reasonably considered representative of our Mexican population from Mexico City. We used a family-based study design with a large number of case-parent triads and we were able to infer haplotypes across the gene at a high rate and test the common haplotypes (frequency >5%) for association with asthma. Our triad design and analysis protects against population stratification and provides estimates of the magnitude of associations (relative risks) in addition to tests of significance. The numbers of the informative families for the two SNPs associated with asthma were high and we were able to examine the effect of the homozygote for the minor allele.

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Our asthma cases were diagnosed by pediatric allergists at a pediatric allergy specialty clinic of a large public hospital. Referral to this pediatric allergy clinic is a tertiary referral, and thus the children in our study had already been seen by a generalist and a pediatrician over time for recurrent asthma symptoms. Diagnoses were made on clinical grounds according to previous guidelines.[15] We did not have tests of bronchial hyperreactivity (BHR). However, physician diagnosis of asthma is a valid outcome compared to objective measurements.[39] Of note, physician diagnosis of asthma has been used as the major endpoint in linkage studies resulting in the positional cloning of asthma genes.[40] We had objective data on atopy; skin prick tests revealed the vast majority of these children with asthma (92%) to be atopic to aeroallergens.

In a large case-parent triad study of a Mexican childhood asthma population, we found that the rs1154404 and rs28730619 SNPs and the most common haplotype across the *GSNOR* gene were associated with childhood asthma risk. Although we have data from only one population, given the strong biological rationale for an important role of GSNOR in asthma pathogenesis, these findings are of interest.

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Abbreviations

ADH5
: alcohol dehydrogenase 5

CI
: confidence interval

FDH
: formaldehyde dehydrogenase

FEV_1
: forced expiratory volume in one second

GSNO
: S-nitrosoglutathione

GSNOR
: S-nitrosoglutathione reductase

NO
: nitric oxide

RR
: relative risk

SNO
: S-nitrosothiol

SNP
: single nucleotide polymorphism

TDT
: transmission disequilibrium test

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Table I

Demographic and clinical characteristics of the 532 asthmatic children.

Clinical characteristics	%
Mean age (SD), yr	9.0 (2.4)
Sex (male)	58.8
Asthma severity[a]	
Mild	71.7
Moderate to severe	28.3
Asthma Medication in the past 12 mo[b]	98.1
FEV_1, mean (SD), percent predicted[c]	96.8 (19.7)
Skin test positivity (out of 24 aeroallergens)[d]	
≥ 1 allergen	91.7
≥ 5 allergens	51.6
Parental smoking[e]	
Mother smoked during pregnancy	5.0
Current smoking parent	51.2

[a] Asthma severity data were available for 508 children.

[b] Asthma medication data were available for 526 children.

[c] Pulmonary function data were available for 401 children.

[d] Skin test data were available for 483 children.

[e] Parental smoking data were available for 527 children.

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Table II

Distribution of case-parent triad genotypes for 5 *GSNOR* SNPs.

Mother-father-child[a]	Triad counts				
	rs1154401	rs1154404	rs1154410	rs1154412	rs28730619
222	1	4	0	0	21
212	1	10	0	0	30
211	3	17	0	1	19
122	5	10	0	2	35
121	1	15	1	5	25
201	10	15	4	5	33
021	16	26	5	10	27
112	8	24	4	0	38
111	25	42	5	16	57
110	18	31	5	13	32
101	49	47	31	51	48
100	51	61	39	44	35
011	55	46	32	41	43
010	50	58	39	37	29
000	235	124	357	306	54
Total triads	528	530	522	531	526

[a]Number of copies of minor allele in mother-father-child.

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Table III

GSNOR SNPs in relation to childhood asthma risk.

SNPs	Genotype	Relative risk (95% CI)[a]
rs1154401	GG	1.00
	GC	0.96 (0.74–1.23)
	CC	0.69 (0.37–1.31)
rs1154404	TT	1.00
	TA	0.77 (0.61–0.97)[b]
	AA	0.66 (0.44–0.99)[c]
rs1154410	CC	1.00
	CT	0.78 (0.57–1.08)
	TT	1.03 (0.32–3.33)
rs1154412	GG	1.00
	GA	1.04 (0.79–1.37)
	AA	0.64 (0.27–1.51)
rs28730619	AA	1.00
	AG	1.20 (0.92–1.55)
	GG	1.60 (1.13–2.26)[d]

[a]Homozygotes of major allele are the reference category.

[b]P = 0.028; false discovery rate = 0.06.

[c]P = 0.046; false discovery rate = 0.07.

[d]P = 0.0077; false discovery rate = 0.03.

For FBAT users, P values from the additive model were 0.4 for rs1154401, 0.015 for rs1154404, 0.17 for rs1154410, 0.80 for rs1154412, and 0.0084 for rs28730619.

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Table IV

GSNOR haplotypes in relation to childhood asthma risk.

Haplotype[a]	Frequency	Relative risk (95% CI)	
		Single Copy	Double Copy
ht1 (GTCGG)	0.418	1.14 (0.87–1.51)	1.57 (1.09–2.30)[b]
ht2 (GACGA)	0.328	0.84 (0.65–1.10)	0.80 (0.52–1.21)
ht3 (CTCAA)	0.073	1.19 (0.84–1.67)	0.45 (0.06–3.26)
ht4 (CTTAA)	0.061	0.91 (0.62–1.34)	0.64 (0.08–4.45)
ht5 (GTCGA)	0.057	1.13 (0.76–1.67)	0.62 (0.08–4.99)

[a]The haplotypes formed by rs1154401G>C, rs1154404T>A, rs1154410C>T, rs1154412G>A, rs28730619A>G in order.

[b]P = 0.017; false discovery rate = 0.02. For FBAT users, the P value from the additive model was 0.011.