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Genetic variants and risk of asthma in an American Indian population

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ABSTRACT

Background: Asthma is recognized as a complex, multifactorial disease with a genetic component that is well recognized. Certain genetic variants are associated with asthma in a number of populations.
Objective: To determine whether the same variants increase the risk of asthma among American Indian children.
Methods: The electronic medical records of an Indian Health Service facility identified all children between 6 and 17 years of age with case-defining criteria for asthma (n = 108). Control children (n = 216), matched for age, were also identified. Real-time polymerase chain reaction assays were used to genotype 10 single-nucleotide polymorphisms (SNPs) at 6 genetic loci. Genotypic distributions among cases and controls were evaluated by χ^2 and logistic regression methods.
Results: A variant at 5q22.1 revealed a statistically significant imbalance in the distribution of genotypes between case-control pairs (rs10056340, $P < .001$). In logistic regression analyses, the same variant at 5q22.1 and a variant at 17q21 were associated with asthma at $P < .05$ (rs10056340 and rs9303277). Inclusions of age, body mass index, and atopy in multivariate models revealed significant associations between rs10056340 (odds ratio, 2.020; 95% confidence interval, 1.283–3.180; $P = .002$) and all 5 17q21 SNPs and asthma in this population. In analyses restricted to atopic individuals, the association of rs10056340 was essentially unchanged, whereas among nonatopic individuals the trend was in the same direction but nonsignificant. The reverse was true for the 17q21 SNPs.
Conclusion: These findings demonstrate that many variants commonly associated with asthma in other populations also accompany this condition among American Indian children. American Indian children also appear to have an increased risk of asthma associated with obesity.

Introduction

The pathogenesis of asthma is multifactorial, including genetic, environmental, and social factors and the interaction between them (gene-environment interactions).[1] A further challenge facing investigations of this condition is that there are likely multiple phenotypes or subtypes of what has been clinically diagnosed as asthma[2–5] and potential heterogeneity in risk factors for asthma between racial/ethnic groups.

Clinicians have long recognized the increased risk of asthma in certain families, and genetic epidemiologic studies have calculated heritabilities of 35% to 95%.[6,7] Twin studies provide similar support for strong heritability, with concordance rates among monozygotic twins being approximately 75% compared with 35% among dizygotic twins.[8] Although there are variable results, depending on methods and temporal factors, many studies have found that asthma is more prevalent in the American Indian (AI) population when compared with all other races,[9–12] but little is known about specific causes or phenotypes. One study found that AIs have the highest asthma rate among single-race groups, with 18.5% of AIs diagnosed with asthma, whereas only 11% are diagnosed with asthma among the US adult population.[9]

There are several, well-replicated genetic variants that have been previously associated with asthma, notably variants at 17q21.[7,13–17] Although associated single-nucleotide polymorphisms

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(SNPs) in this region encompass at least a 380-Kb span,[7] including 5 annotated genes,[13,18] initial attention has centered primarily on *ORMDL3* and *GSDMB*. Calcium homeostasis, sphingolipid metabolism, and lymphocyte function are affected by variants that influence expression of *ORMDL3*,[19–22] whereas *GSDMB* is highly expressed in T lymphocytes and thus potentially affects the inflammatory response, particularly to viral infection.[16,23] Calcium sensing and regulation have recently been found to have a profound influence on airway reactivity and suggest therapeutic targets for asthma control.[24] Additional genetic variants with a robust association with asthma phenotypes have been found at 2q12.1,[14,17,25] 5q22.1,[25] 5q31.1,[14,15,17] 7q22.3,[14] 9p24.1,[14,15] and 11q13.5.[25] The function of candidate genes at these loci are involved in a myriad of functions,[26] including cytokine regulation of inflammatory cells (*TSLP, IL1RL1, TMEM182, C11orf30, IL33, DPP10*), controlling cellular maturation and differentiation (*HLA-DQB1, CDHR3*), and DNA repair (*RAD50*), and associated with various inflammatory conditions, such as eosinophilic esophagitis,[27] inflammatory bowel disease,[28] and allergic rhinitis.[25]

Although variants at these 6 genetic loci have been replicated across independent studies, most have been implicated solely in individuals with European ancestry. Thus, it is unknown whether these variants represent universal genetic risk factors for asthma across human populations, and in many instances replication attempts have failed when made in a different racial/ethnic group. Furthermore, none of these studies have included AI populations; thus, it is unknown whether similar genetic risk factors are present in this population. Our objective was to investigate the contribution of genetic variants at 6 previously implicated asthma-associated loci in the development of asthma in AI children.

Methods

This analysis derives from a case-control study of the environmental and genetic influences on risk of asthma among an AI population in the northcentral United States. Most primary medical care for this community of predominantly tribal members is provided mainly by federal funding to the Indian Health Service (IHS) and a tribal health department.

The population is located in the northcentral portion of South Dakota in an area covering 4,266 square miles. The area's population is approximately 8,500, giving a population density of between 2 and 3 people per square mile. Most live in cluster housing near small towns or in cluster sites far removed from basic services. Beyond federally supported work in health care and education, ranching and farming provide the bulk of employment. Two counties in this area have 33% and 42% of residents with incomes below the poverty line, making them the 11th and 4th lowest per capita income counties in America, respectively. In addition, more than 20% have less than a high school education.[29]

Cases were ascertained through automated query of the IHS electronic medical records system, searching for an inclusive array of *International Classification of Diseases, Ninth Revision* (*ICD-9*) codes between 493.00 and 493.92, in addition to codes 786.07 (wheezing) and V17.5 (family history of asthma). The search was limited to individuals 6 through 17 years of age. Additional cases were sought by contact with local non-IHS health care professionals. This identified more than 900 individuals, who then gave consent for further review of medical records to determine potential eligibility.

Case definition criteria required (1) a diagnosis of asthma on at least 2 occasions by more than one health care professional during the past 2 years and (2) refills of asthma treatment medications on at least 2 occasions during the past 2 years. Exclusionary criteria were (1) birth weight less than 2,500 g; (2) neonatal ventilator treatment; (3) hospitalization at birth greater than 15 days; (4) congenital heart anomaly requiring surgery; (5) diagnosis of cystic fibrosis; (6) congenital lung, diaphragm, chest wall, or airway anomaly; (7) diagnosis of pneumonia, pertussis, or tuberculosis within the past year; and (8) congenital muscular disorder.

Many of the potential cases initially identified had been assigned an *ICD-9* code that indicated asthma by the pharmacist filling a prescription for a bronchodilator, although the prescribing physician had not indicated a diagnosis of typical asthma and was intending to ameliorate the bronchospastic component of a pulmonary infection. These children did not meet diagnostic criteria but required considerable recruiter effort to contact parents and determine their status via medical record review.

For each case, 2 controls were initially recruited by identifying the 2 children born the day after and before the index case and contacting the parents for consent to review medical records for possible inclusion. As the study progressed, this method did not yield sufficient controls, and many controls were later recruited from previously identified families with children born almost exclusively (>99%) within 6 months of the index case. Initial recruitment was concentrated on cases and later focused on controls, which resulted in a slight bias toward controls being older at the time of examination (even though birth dates were generally within protocol limits). Nonetheless, all but 5 (2.3%) of the pairs were examined within 1 year of each other. Controls met the same exclusionary criteria as cases in addition to (1) no diagnosis of asthma by any health care professional during the past 2 years and (2) no prescriptions of any asthma medications during the past 2 years.

Consenting cases and controls were then examined according to study protocol, which included anthropomorphic measures, spirometry, salivary DNA collection, and a nonfasting blood draw. Environmental measures of home air quality and dust exposure were made.

A questionnaire collected social, demographic, and medical history from cases and controls, most of which will be reported in the future. One question—"Has a medical person ever said that your child had hay fever or seasonal allergies?"—sought to gauge atopic symptoms. Total white blood cell (WBC) count, percentage of eosinophils, and serum measures of high-sensitivity C-reactive protein, total IgE, and specific IgE reactive to 5 airborne antigens (dog and cat dander, dust mite, cockroach, and Alternaria mold) were assessed as covariates for analysis. A specific IgE antibody titer above the detection limit to at least 1 of the above 5 aeroallergens was defined as atopy. Salivary cotinine levels were used to adjust for tobacco smoke exposure because self-reported exposure is subject to bias, especially considering public recognition of adverse effects for children with asthma and for some reluctance of minors to admitting of smoking behavior.

Genetic variants previously associated with asthma risk were chosen from the literature as indicated and referenced in the introduction. Genomic DNA was collected and extracted from salivary samples using the Oragene (DNA Genotek Inc, Ontario, Canada) system and the manufacturer's directions. Predesigned TaqMan (Applied Biosystems Inc, Foster City, California) genotyping assays and protocols were implemented for SNPs on a real-time Mini-Opticon (Bio-Rad Laboratories Inc, Hercules, California) 4-color thermocycler. Positive controls were identified for each of the 3 possible genotypes for each SNP and included with no template controls in each genotyping assay. There were 11 samples that consistently failed genotyping attempts, probably because of failed preservation. The number of case-control pairs analyzed varied slightly by SNP because primer reagents were occasionally exhausted and it was not cost-efficient to reorder primers for a small number of additional samples.

Clinical, environmental, and genetic comparisons between asthma cases and controls were performed using a McNemar

Table 1
Characteristics of SNPs Studied and Study Population Prevalences (Cases and Controls)

dbSNP ID	Chromosome band	Nearest gene	Annotation	Alleles (major/minor)	Minor allele frequency (95% CI), %[a]	Hardy-Weinberg *P* value
rs2305480	17q21	*GSDMB*	Missense	G/A	35.0 (31.2–38.7)	.67
rs7216389	17q21	*GSDMB*	Intronic	T/C	39.6 (35.8–43.4)	.87
rs8076131	17q21	*ORMDL3*	Intronic	A/G	34.4 (30.7–38.2)	.51
rs4795405	17q21	*LRRC3C*	Intronic	C/T	37.9 (34.1–41.7)	.79
rs9303277	17q21	*IKZF3*	Intronic	C/T	39.0 (35.1–42.9)	.70
rs1558641	2q11.2	*IL1R1*	5′ Upstream	G/A	4.6 (3.0–6.3)	.09
rs10056340	5q22.1	*SLC25A46-TSLP*	Intergenic	T/G	16.5 (13.6–19.3)	.32
rs6871536	5q31	*RAD50*	Intronic	T/C	15.2 (12.4–18.0)	.60
rs928413	9q21	*IL33*	5′ Upstream	A/G	8.4 (6.2–10.5)	.54
rs2155219	11q13.4	*EMSY-LRRC32*	Intergenic	G/T	47.3 (43.4–51.2)	.78

Abbreviations: CI, confidence interval; ID, identification; SNP, single-nucleotide polymorphism.
[a]Minor allele frequency among cases and controls combined.

χ^2 test for discrete variables and a paired *t* test for continuous variables. Univariate and multivariate logistic regression was used to explore the effects of genotype and covariates on risk of asthma under an additive model. All models were adjusted for age because there was a significant difference between cases and controls attributable to factors noted previously. Descriptive analyses were performed using SPSS statistical software, version 13.0.1 (IBM Inc, Armonk, New York). Logistic regression was performed using LogXact-11 (Cytel Inc, Cambridge, Massachusetts).

Results

All 10 variants previously found to be associated with asthma in populations of European ancestry were polymorphic in this AI population in northcentral United States. Table 1 provides an orientation to the 10 genetic variants analyzed here, their frequency in the combined cases and controls, and results of tests for Hardy-Weinberg equilibrium. The prevalence of these alleles in other populations can be seen in Figure 1.

The characteristics of cases and controls are listed and compared in Table 2. Asthma controls were significantly older than asthma cases, likely because of several factors related to recruitment protocols, as noted in the Methods section. The mean difference in age is approximately 4 months older for the controls, and for this reason, all logistic regression models were adjusted for age. Asthma cases had significantly higher body mass index (BMI), total serum IgE, and WBC count compared with asthma controls, and atopy was more prevalent among the cases (Table 2). High-sensitivity C-reactive protein was not significantly different between cases and controls.

An analysis of genotypes discordant between case and control pairs is summarized in Table 3. One SNP at 5q22.1, rs10056340, had a significant association with case-control status after Bonferroni adjustment for 10 comparisons ($\alpha_{10} = .005$, $P < .001$), considering the major or minor allele in a dominant model. One SNP at 17q21 was associated at $P = .03$ (rs9303277), and 3 additional SNPs were suggestive at $P < .10$ (rs2305480, rs7216389, and rs8076131), considering the major allele in a dominant model.

Results of primarily univariate logistic regression analysis of clinical and environmental covariates are presented in the eTable 1. Because age was slightly more advanced among controls compared with cases, all univariate analyses were adjusted for age. The demographic and immune measure covariates of BMI, WBC, eosinophils, total IgE, and atopy were all significantly associated

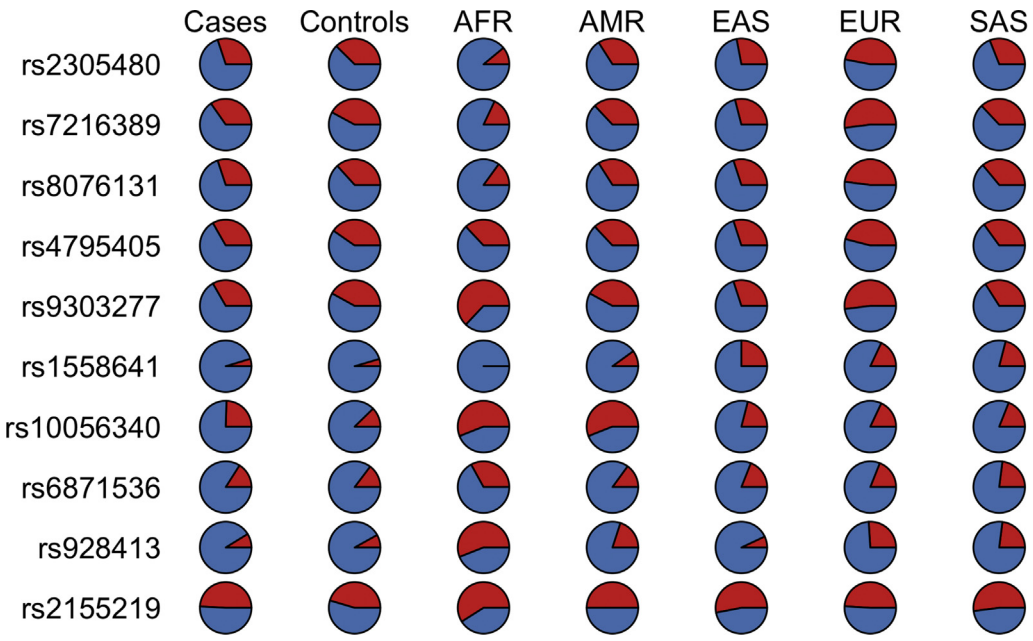

Figure 1. Prevalence of genotyped single-nucleotide polymorphisms in cases and controls of the present study and selected global populations. Minor allele (Table 1) is shown in red. The populations are indicated by standard 1000 Genomes Project notation. AFR, African; AMR, admixed American; EAS, East Asian; EUR, European; and SAS South Asian.

Table 2
Characteristics of Matched Cases and Controls[a]

Characteristic	Cases (n = 108)	Controls (n = 215)	*P* value
Male sex	57 (52.8)	109 (50.7)	.773[b]
Age, mean (SD), y	11.80 (3.22)	12.14 (3.20)	<.001[c]
BMI, mean (SD)	25.43 (8.16)	23.59 (6.60)	.005[d]
WBC count, mean (SD), $\times 10^9$/L	7.54 (2.38)	6.88 (1.82)	.017
Eosinophils, %	4.99	3.79	.931
CRP, mean (SD), mg/L	2.17 (2.66)	1.72 (2.21)	.173
Total IgE, mean (SD), kU/L	486.4 (705.2)	219.3 (371.3)	<.001
>1 Antibody over detection limit	66 (61)	61 (28)	<.001
rs2305480, 17q21, A[e]	65/216 (30.1)	154/410 (37.6)	.076[f]
rs7216389, 17q21, C	75/216 (34.7)	176/418 (42.1)	.086
rs8076131, 17q21, G	65/216 (30.1)	152/414 (36.7)	.116
rs4795405, 17q21, T	72/216 (33.3)	166/412 (40.3)	.105
rs9303277, 17q21, T	70/210 (33.3)	168/400 (42.0)	.046
rs1558641, 2q11.2, A	9/214 (4.7)	19/414 (4.6)	.987
rs10056340, 5q22.1, G	53/216 (24.5)	51/416 (12.3)	<.001
rs6871536, 5q31, C	34/212 (16.0)	61/414 (14.7)	.755
rs928413, 9q21, G	19/216 (8.8)	33/406 (8.1)	.893
rs2155219, 11q13.4, T	106/216 (49.1)	187/412 (45.4)	.426

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CRP, C-reactive protein; WBC, white blood cell.
[a]Data are presented as number (percentage) unless otherwise indicated.
[b]Differences between discrete variables evaluated with the McNemar χ^2 test.
[c]Differences between means evaluated with paired *t* test, see Results section for explanation of the significant difference in age.
[d]*P* value for all continuous variables based on analysis of natural log transformed values.
[e]Frequency of designated allele; 65/216 indicates 65 minor alleles (A in this case) and 151 major alleles for a total of 216.
[f]Differences in allele frequency evaluated with the χ^2 test.

with case status in univariate logistic analysis. C-reactive protein levels did not appear to be associated with asthma in this analysis. Although the nonsignificant estimate of the salivary cotinine level was in the direction of a protective effect, this may be because of parents properly limiting exposure of their children with asthma to tobacco smoke. Self-reported tobacco smoke exposure was not significantly associated with asthma in univariate analysis for any of 4 different questionnaire responses (minimum $P = .55$).

Multivariate logistic regression was then used to assess each SNP in an additive genetic model, adjusting for age, BMI, and atopy. Although BMI became attenuated below $P = .05$ when multivariate models included WBC count, the 2 measures were strongly correlated (Pearson coefficient = 0.351, $P < .001$), and BMI was believed to be the primary determinant of the 2 measures. The results of these analyses are found in Table 4. rs10056340 was significantly associated with asthma in the multivariate model after Bonferroni correction for 10 comparisons, and all 5 17q21 SNPs have associated with asthma at $P < .05$ after adjustment for these 3 covariates. No individual 17q21 SNP is statistically significant after a conservative Bonferroni correction for 10 comparisons. However, it is only expected to identify 1 SNP at $P < .05$ by chance given 10 comparisons, and 5 are observed, demonstrating an enrichment of true-positive associations with asthma at 17q21.

To further examine the interaction of atopy and BMI with these SNPs and risk of asthma, we performed stratified analyses according to atopy and BMI. Among atopic individuals (adjusting for age and BMI), the association remained statistically significant for rs10056340 (odds ratio [OR], 2.620; 95% confidence interval [CI], 1.334–5.149; $P = .005$), whereas among nonatopic individuals, the results were in the same direction (OR, 1.561; 95% CI, 0.817–2.980; $P = .18$). The converse was true for the 5 SNPs at 17q21, which revealed an association at $P < .05$ among nonatopic individuals but no association among atopic individuals. The absolute number of circulating eosinophils (ABS/EOS) was highly correlated with atopy status and cumulative number of specific IgEs, showing sensitization. Substitution of ABS/EOS for atopy in logistic models gave similar results, but when atopy was added to the model, ABS/EOS became nonsignificant. The same pattern of interaction between atopy as it relates to rs10056340 and the 17q21 SNPs was seen when the ABS/EOS variable was substituted for atopy. Similarly, the leukocyte count lost significance if included in models with the primary covariates listed in Table 4, and final analyses including representative 5q22.1 and 17q21 SNPs were unchanged.

Stratifying on BMI, rs10056340 had a similar trend in those below and above the median natural logarithm of BMI ($P = .02$ and .07, respectively, adjusting for age, natural logarithm of BMI, and atopy). Four of the 5 17q21 SNPs also had nominally significant association but only in analyses limited to those with natural logarithm of BMI below the median (maximum $P < .048$).

Fully adjusted models, including age, natural logarithm of BMI, and atopy status, had no significant interaction between rs10056340 or rs2305480 (the prototypic 17q21 SNP) and any of the environmental factors listed in Table 4.

Discussion

We found that 10 common genetic variants previously associated with asthma in other racial/ethnic groups are polymorphic in a Northern Plains AI population, and 6 of those variants are similarly associated with asthma at 5q22.1 and 17q21. Our results indicate the presence of shared genetic risk factors for asthma in the Northern Plains AI population with other racial/ethnic groups at 5q22.1 and 17q21. The fact that these associations have been confirmed in an AI population lends further credence to the likely functional effects of these variants and/or the existence of an extended haplotype, which is shared between AI and more distantly related populations.

Table 3
Genotypes Associated With Case-Control (Matched-Pair) Status

dbSNP	Discordant pairs with dominant major allele				Discordant pairs with dominant minor allele				Total no. of matched pairs
	Allele	No. of pairs	No. of alternate situations[a]	*P* Value	Allele	No. of pairs	No. of alternate situations[a]	*P* value	
rs2305480, 17q21	G	15	28	.07	A	39	55	.12	205
rs7216389, 17q21	T	20	34	.08	C	39	55	.12	209
rs8076131, 17q21	A	15	28	.07	G	41	53	.26	207
rs4795405, 17q21	C	18	30	.11	T	37	53	.11	206
rs9303277, 17q21	C	17	33	.03	T	37	47	.33	194
rs1558641, 2q11.2	G	0	2	NA	A	14	15	>.99	205
rs10056340, 5q22.1	G	18	2	<.001	T	67	30	<.001	208
rs6871536, 5q31	T	6	3	.51	C	43	40	.83	203
rs928413, 9q21	A	2	2	>.99	G	29	26	.79	203
rs2155219, 11q13.4	G	47	31	.09	T	46	37	.380	206

Abbreviation: NA, not applicable.
[a]Number of alternate situations in which the control genotype is dominant for the major allele.

Table 4
Multivariate Logistic Regression Analysis of Factors Associated With Asthma

Characteristic	OR (95% CI)	*P* value
Age	0.909 (0.833–0.992)	.03
Body mass index	3.219 (1.204–8.610)	.02
>1 specific antibody over detection limit	3.889 (2.370–6.381)	<.001
Above 3 covariates plus genetic variants		
rs2305480, 17q21, A allele	0.635 (0.434–0.927)	.02
rs7216389, 17q21, C allele	0.681 (0.472–0.982)	.04
rs8076131, 17q21, G allele	0.668 (0.459–0.971)	.04
rs4795405, 17q21, T allele	0.680 (0.469–0.985)	.04
rs9303277, 17q21, T allele	0.648 (0.447–0.940)	.02
rs1558641, 2q11.2, A allele	1.044 (0.457–2.386)	.92
rs10056340, 5q22.1, G allele	2.020 (1.283–3.180)	.002
rs6871536, 5q31, C allele	0.968 (0.591–1.585)	.90
rs928413, 9q21, G allele	1.187 (0.634–2.222)	.59
rs2155219, 11q13.4, T allele	1.206 (0.842–1.729)	.31

The association between many of these SNPs and asthma risk may depend on the presence of atopy, with associations at 17q21 SNPs stronger in nonatopic individuals, and association with rs10056340 at 5q22.1, which is stronger among atopic individuals. Similarly, the influence of the 17q21 SNPs on risk of asthma may also show interaction with BMI, with an association seen primarily among those below the median natural logarithm of BMI. However, these observations are largely qualitative, given that the differences observed may reflect the reduction in power from a reduced sample size in a stratified analysis. Nevertheless, without adjustment for atopy or BMI in our logistic model, associations with SNPs at 17q21 become nonsignificant. Of note, the association between asthma and obesity has been seen previously in AI[30] and other populations.[31,32]

Our most significant association was with a noncoding variant 3′ downstream of *SLC25A46* and 5′ upstream of *TSLP* (rs10056340 at 5q22.1). SLC25A46 is a mitochondrial solute carrier protein involved in mitochondrial fission[33]; however, a functional role of this protein in asthma remains to be seen. TSLP promotes cellular response of T_H2, which contributes to many inflammatory diseases, including asthma, allergy, and chronic obstructive pulmonary disease. Variation at 5q22.1 has been associated with asthma in a meta-analysis of African Americans, Hispanic/Latinos, and European Americans, with evidence of association in all 3 racial/ethnic groups.[34] Variants at 5q22.1, including rs10056340, have been previously associated with allergic sensitization in individuals with European ancestry[25,35] and with atopic dermatitis in the Chinese Han population.[36] The association between asthma and allergic disease has been well established[37,38]; thus, the association at rs10056340 and asthma in Northern Plains AIs suggests atopic asthma is an important subphenotype present in this population. We further note the association between SNPs at 17q21 and asthma was sensitive to adjustment for the presence of atopy, which further emphasizes the importance of considering asthma subphenotypes in genetic analyses.

Variants at 17q21 have consistently been associated with asthma in prior studies, and our results indicate the importance of this locus and asthma susceptibility in Northern Plains AIs. This is arguably the most well-replicated asthma locus, including *ORMDL3* and *GSMDB*, with established associations in individuals with European ancestry,[39,40] Slovenians,[41] Chinese residents of Singapore,[42] Pakastanis,[43] Koreans,[44] African Americans,[45] Puerto Ricans, and Mexicans.[45] Variants at this locus are most strongly associated with childhood asthma, and given that participants in our study were between the ages of 6 and 17 years, we may have expected a stronger signal of genetic association. However, because of the presence of high linkage disequilibrium and the resulting long haplotypes at 17q21, causal variants remain to be identified and may not have been directly genotyped in our study. Thus, additional studies in AI populations are necessary to identify causal variants at 17q21, including genotyping a broader spectrum of variants or direct sequencing in a greater number of individuals.

Genetic associations from additional variants queried, including those at 2q12 (*IL1R1*), 5q31 (*RAD50*), 9p24, and 11q13, could not be established despite being polymorphic and sufficiently common in our study population. This finding may indicate differences in the genetic architecture of asthma in Northern Plains AIs at these loci, differences in linkage disequilibrium with the underlying causal variants (assuming the variants typed themselves are not causal), or insufficient statistical power to establish a significant association. Furthermore, differences in environmental exposures may also play a role, which can modify the effect of a genetic variant on the risk of asthma (ie, gene-environment interactions). Environmental factors are known to play an important role in asthma, for example, socioeconomic status, farming, exposure to tobacco smoke, and air pollution. Thus, additional studies are required to fully exclude the possibility that variants at these loci do not contribute to asthma susceptibility in Northern Plains AIs.

A major strength of our study is the population-based ascertainment of cases and controls in a Northern Plains AI community—a population with high rates of asthma—yet this is notably absent in prior genetic studies of asthma. Unfortunately, for historical reasons, AI communities have occasionally taken a skeptical approach to biomedical research, in particular, genetic studies. The present investigation benefited from collaboration with a local research organization with a long and deep presence and commitment to the community. Additional strengths include the use of electronic medical records to establish case-control criteria and the availability of important environmental and clinical covariates, including atopy, for more detailed analyses.

Overall, our findings reveal the importance of genetic variants at 5q22.1 and 17q21 with risk of asthma in an AI population. This is an important step in enhancing our understanding of the pathophysiology of asthma in a vulnerable population that has been historically absent in genetic studies of asthma. Identifying the spectrum of genes and genetic loci that are relevant to asthma, specifically in AI children, will facilitate the translation of novel therapeutics and preventive strategies that result from the genomics era of precision medicine.

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Supplementary Data

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eTable 1
Logistic Regression Analysis With Adjustment for Age Attributable to Significant Age Difference Between Cases and Controls[a]

Characteristic	OR (95% CI)	*P* value
Age	0.965 (0.897–1.038)	.34
Male sex	1.072 (0.674–1.706)	.77
Body mass index	3.728 (1.460–9.516)	.006
White blood cell count	3.030 (1.267–7.248)	.01
Eosinophils	1.504 (1.092–2.071)	.01
CRP	1.199 (0.956–1.504)	.12
IgE (total)	1.422 (1.200–1.686)	<.001
>1 specific antibody over detection limit	4.047 (2.478–6.609)	<.001
Environmental variables		
Saliva cotinine category	0.781 (0.578–1.054)	.11
Exhaled carbon monoxide	1.043 (0.895–1.216)	.59
PM_1 level	4.933 (0.985–24.714)	.052
$PM_{2.5}$ level	3.879 (0.915–16.443)	.07
PM_4 level	4.307 (1.053–17.612)	.04
PM_{10} level	6.389 (1.340–30.468)	.02
Indoor temperature	0.988 (0.940–1.038)	.64
Outdoor temperature	0.984 (0.974–0.994)	.003
Indoor relative humidity	0.974 (0.958–0.990)	.002
Outdoor relative humidity	0.999 (0.987–1.011)	.90
Indoor carbon dioxide	1.000 (1.000–1.001)	.10
Indoor carbon monoxide	1.043 (0.895–1.216)	.59
Home heating category	1.096 (0.862–1.395)	.45

Abbreviations: CI, confidence interval; CRP, C-reactive protein; OR, odds ratio; PM, particulate matter.
[a]The ORs indicate increased risk associated with a 1-unit change in the natural log of continuous variables.