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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