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Genetic Approach Identifies Distinct Asthma Pathways In Overweight vs. Normal Weight Children

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Abstract

Background—The pathogenesis of asthma in the context of excess body weight may be distinct from asthma that develops in normal weight children. The study's objective was to explore the biology of asthma in the context of obesity and normal weight status using genetic methodologies.

Methods—SNP associations with asthma and interactions between SNPs and overweight status in 49 genes were assessed in child-participants of the Greater Cincinnati Pediatric Clinic Repository.

Results—Asthma was significantly associated with weight (OR=1.38; p=0.037). The number of genes and the magnitude of their associations with asthma were notably greater when considering overweight children alone versus normal weight and overweight children together. When considering weight, distinct sets of asthma-associated genes were observed, many times with opposing effects.

Conclusions—We demonstrated that the underlying heterogeneity of asthma is likely due in part to distinct pathogenetic pathways that depend on preceding/co-morbid overweight and/or allergy. It is therefore important to consider both obesity and asthma when conducting studies of asthma.

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Conflict of Interest Statement

There are no conflicts of interests to report.

Author Contributions

Project conception: MBK, LJM; Project design: MBK, JMBM, GKKH; Data storage and electronic medical record query: ML, MBK; Data manipulation and statistical analysis: HH, LJM, MBK; Data interpretation: MBK, JMBM, LJM, GKKH; Manuscript preparation: MBK, LJM, JMBM, GKKH, HH; Ingenuity Pathway Analysis: TBM, HJ; Final approval of the manuscript: MBK, LJM, JMBM, GKKH, HH, TBM, HJ, ML.

Keywords

allergy; epidemiology; interaction; overweight; children

Introduction

The factors contributing to asthma heterogeneity are not well understood. Increasing weight of asthmatics may explain some of the heterogeneity (1-3). The National Health and Nutrition Examination Survey revealed significant asthma associations with obesity regardless of allergic status (4). Few studies have observed associations between obesity and allergic status suggesting that each uniquely contributes to asthma risk. Several recent studies of asthma (versus non-asthma) and obesity have been reported (5-7), although none have examined the genetic patterns stratified by weight and allergic status. We addressed this gap in the current literature.

Methods

Recruitment and Genotyping

This study was approved by Cincinnati Children's Hospital Medical Center (CCHMC) Institutional Review Board. Verbal and written consent was obtained from parent(s)/authorized representatives. Included were Caucasians, ages 4-17 years from the Greater Cincinnati Pediatric Clinic Repository (GCPCR) and CCHMC Genomic Control Cohort (GCC) (8). Given that GCPCR enrolls primarily from hospital allergy and pulmonary clinics, the population is enriched with asthmatics, all diagnosed per American Thoracic Society criteria (9). Weight status was based on height and weight measurements taken closest to the consent date. Children with 85th BMI percentiles (considering age and sex based on the Centers for Disease Control and Prevention curves) were considered overweight (10). Asthmatic GCPCR participants with allergic rhinitis (AR), atopic dermatitis (AD), or SPT+ to 1 allergen (8) were considered allergic asthmatics. SPT-asthmatics without AR or AD were considered non-allergic asthmatics. Non-asthmatic GCPCR children with AR and/or AD or non-asthmatic GCC children who reported ever having environmental allergies, hayfever or eczema were considered allergic controls. Non-allergic control children were those not having any personal or family history of asthma, and no history of hayfever, environmental allergies, AR, or AD.

Genomic DNA was isolated from saliva, buccal or blood samples (8) and genotyped using a custom Illumina Golden Gate assay (San Diego, CA). As previously described, the assay included 716 tagging single nucleotide polymorphisms (SNPs), 18 non-synonymous SNPs and four promoter SNPs within 52 candidate genes (11, 12). These candidate genes were chosen based on a high number of replications reported in the literature (>10) and biologic relevance in the pathogenesis of asthma or allergy. Specifically, they are located in pathways related to the epithelium, immunity, inflammation, oxidative stress, microbe sensing and/or protease inhibition. In total, 750 asthmatics, 419 non-asthmatic/allergic and 349 non-asthmatic/non-allergic children were genotyped. Tagging SNPs were selected using Haploview and Tagger (<http://www.broad.mit.edu/mpg/haploview>) (11, 12) and 30

ancestry-informative markers (AIMs) were included(13). Genotypes were assigned using BeadStudio v3.2 (San Diego, CA). SNP call rate was >94.74%. X chromosome SNPs (N=22) were not included in the analysis. SNPs failing Hardy-Weinberg Equilibrium in the non-allergic controls ($p<0.0001$), with missing call rates >10%, or MAF <10% were removed from the analysis (N=233). Therefore, a total of 491 SNPs remained in the analysis plus 22 AIMs available for principal component analysis. Twelve subjects with >20% total SNPs missing were also excluded.

Analysis

Principal component (PC) analysis was performed on AIMs using EIGENSTRAT (14, 15) to account for population stratification. Genomic inflation factors were estimated as 1 after PC adjustments. Assuming an additive genetic model, asthma associations were tested after adjusting for age, sex and PCs using the whole cohort as well as stratified by weight status. Logistic regression was also used to test for evidence of interactions between SNPs and weight status while accounting for the main SNP effect, weight status, age, sex and PCs. To determine a family wise error rate, we permuted asthma - weight status 1000 times and performed interaction analyses for each SNP (1000 permutations x 491 SNPs) and examined their distributions. We then identified 0.049 as the nominal significance threshold. To determine if weight status influenced genetic associations with asthma, we examined the effect size of the associations from the entire cohort to those stratified by weight. By examining the effect size, we sought to overcome power issues in detecting interaction effects. Because the 52 genes were selected based on their biologic relevance in asthma, we expected that this sample would be enriched for genetic effects. If gene-environment interactions existed, the effects (odds ratios) in the combined analyses would be weaker than in stratified analyses. To test this, the median absolute effect sizes across all variants were compared from the combined and weight-stratified analyses using Wilcoxon paired sign rank test. To determine if the number of genes associated differed by comparison group, we adjusted for the differences in sample size of our comparison groups by identifying the odds ratio (OR) for which there was 80% power to detect associations at $\alpha=0.05$ and MAF=0.2 in the full cohort. Secondary analyses were performed using 3 sets of controls (all, allergic controls, and non-allergic controls). OR thresholds of 1.40, 1.485 and 1.50 were used for asthma vs. all non-asthmatics, non-allergic, allergic children, respectively. Analyses were performed in PLINK.

Results

Children with both genotyping and weight status data (N= 828) were included (Table 1). Asthma was significantly associated with overweight status ($p=0.037$; OR=1.38). There were more allergic asthmatics than allergic non-asthmatics ($p<0.0001$), and the proportion of allergic children was slightly greater among the overweight groups (69.5% vs.62.8%, $p=0.066$; Table 1).

Interaction analyses indicated that 19 SNPs in 10 genes exhibited nominally significant interaction (empiric $p<0.049$) with weight (data not shown), however none were significantly different after a strict correction for multiple testing. Interestingly, these SNPs

effects were often substantially different between the normal and overweight groups in both directionality and magnitude suggesting that overweight may alter the underlying risk for asthma conferred by many genes and obscure the main effects of unstratified or unadjusted analyses.

We examined effect sizes to better understand how weight and allergy status influenced the strength of asthma associations. The effect sizes with asthma for overweight children (median OR=1.145; $p < 1 \times 10^{-15}$) and for normal weight children (median OR=1.097; $p = 1.8 \times 10^{-10}$) were significantly higher than in the full sample (median OR=1.078; Figure 1A). Further, the effect sizes when asthmatics were compared to non-allergic children (median OR=1.109) were significantly higher than when compared to allergic children (median OR=1.085; $p = 3.8 \times 10^{-6}$) and to all non-asthmatics (median OR=1.078; $p < 1 \times 10^{-15}$) (Figure 1A). The median effect size comparing our non-asthmatic control groups (allergic vs. non-allergic) was 0.9981 and there were no statistical differences when these groups were stratified by weight (data not shown).

To determine if the number of genes associated with asthma across the comparison groups differed, we employed an effect size threshold to account for differences in sample sizes (Figure 1B). Overall, more genes were identified in the overweight group. The fewest asthma-associated genes was identified in the full unstratified sample. Of note, while there was some overlap in genes between the normal and overweight group, a substantial number were identified in only one group. Indeed, there was minimal overlap between identified genes when the comparison control group was allergic versus non-allergic. Indeed, only 3 genes identified in the overweight group were common between the normal and overweight groups. These results support that weight and allergic status result in distinct pathways to asthma.

Discussion

The heterogeneity of asthma has been well documented although the factors that underlie it are not well understood (5). Our analysis demonstrates how genetic approaches can be used to investigate the complex interplay between weight and allergic status in asthma pathogenesis. Indeed, through subset and interaction analyses, we found differing patterns of genes associated with asthma, often with opposing effects depending on the underlying presence of overweight and allergic status (Figure 2). Collectively, our data support that both distinct and common pathogenetic pathways promote asthma in the context of being overweight, a finding that may partially explain the heterogeneity in phenotypes observed and the inconsistencies observed in large GWAS of mixed phenotypes. Because different genes and biologic pathways are involved, the effectiveness of clinical treatments likely varies. Knowledge of the pathways driving these specific associations may identify therapeutic targets specifically tailored towards the treatment of asthma in overweight children. Similar future studies, particular in non-Caucasian populations that have reported even higher prevalences of asthma and overweight, are needed to further explore the observed phenomenon.

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Figure 1A **Figure 1B** **Figure 1. Markedly Different Median Effect Sizes and Number of Genes Associated with Asthma by Weight Status**

A) The median of odds ratios (ORs) for the risk variants across 52 genes are shown for all subjects as well as independently for both the normal weight and overweight groups and by allergic group. ORs are compared between groups using the Wilcoxon paired sign rank test.

B) Logistic regression was performed adjusting for sex, age and population stratification. The odds ratio threshold for significance was set where there was 80% power to detect associations at $\alpha = 0.05$ and $MAF = 0.2$ considering the sample size within each comparison group. The OR threshold for the asthma vs. non-asthma comparison is 1.40; 1.50 for the asthma vs. allergic comparison; and 1.485 for the asthma vs. non-allergic comparison. Genes with at least one SNP exceeding this threshold are reported for all subjects and stratified by weight status highlighting those that are both different and in common between weight groups. Included in the analysis for all weights were 338 asthmatics and 490 non-asthmatics (256 non-allergic and 234 allergic). The normal weight analysis included 208 asthmatics and 337 non-asthmatics (184 non-allergic and 153 allergic). The overweight analysis included 130 asthmatics and 153 non-asthmatics (72 non-allergic and 81 allergic).

Primary Genetic Predisposition	BEFORE Asthma Diagnosis	AFTER Asthma Diagnosis
Allergy		<p>90% of Asthmatic Children are Allergic</p>
Allergy		<p>38% of Allergic Asthmatic Children Are Overweight</p>
Asthma		<p>10% of Asthmatic Children are Non-allergic</p>
Obesity		<p>44% of Non-allergic Asthmatic Children Are Overweight</p>

Figure 2. Distinct Asthma Pathways Based on Genetic Predisposition to Weight Status and Allergic Status of the Control Group

Based on our data, overweight and normal weight children can develop asthma through distinct pathways. Preceding allergy may predispose to obesity due to ensuing inflammation, stress, and reduced activity associated with their diagnosis. Alternatively, overweight children can undergo mechanical changes, experience weight-related inflammation, and may be stressed and/or sedentary. Being both allergic and obese genes can uniquely predispose children to asthma. “Allergy” represents children that were either allergic or atopic.

Table 1

Population Characteristics

	All Subjects (n=828)	Asthma (n=338)	Non-asthma (n=490)	P-value	Normal weight (n=545)	Overweight (n=283)	P-value
Age (mean years $\pm$ SD)	10.59 $\pm$ 3.58	9.85 $\pm$ 3.50	11.11 $\pm$ 3.55	4.9e-07	10.60 $\pm$ 3.70	10.58 $\pm$ 3.36	0.95
Sex (male %)	54.5%	56.8%	52.9%	0.29	51.4%	60.4%	0.016
BMI (overweight%)	34.2%	38.5%	31.2%	0.037	-	-	-
Asthma (%)	40.8%	-	-	-	38.2%	45.9%	0.037
Allergic status (N, allergy %)	825, 65.1%	335, 90.4%	490, 47.8%	< 2.2e-16	543, 62.8%	282, 69.5%	0.066

SD – standard deviation; BMI – Center for Disease Control age and sex adjusted body mass indices.