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# A novel locus for exertional dyspnoea in childhood asthma

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## Abstract

Most children diagnosed with asthma have respiratory symptoms such as cough, dyspnoea and wheezing, which are also important markers of overall respiratory function. A decade of genome-wide association studies (GWAS) have investigated genetic susceptibility to asthma itself, but few have focused on important respiratory symptoms that characterise childhood asthma.

Using whole-genome sequencing (WGS) data for 894 asthmatic trios from a Costa Rican cohort, we performed family-based association tests (FBATs) to assess the association between genetic variants and multiple asthma-relevant respiratory phenotypes: cough, phlegm, wheezing, exertional dyspnoea and exertional chest tightness. We tested whether genome-wide significant associations were replicated in two additional studies: 1) 286 asthmatic trios from the Childhood

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Author contributions: S. Lee, C. Lange and J. Hecker conceptualised and designed the project, performed statistical analyses and interpretation, and drafted the manuscript. J.A. Lasky-Su and S.T. Weiss assisted in data preparation and the analyses, as well as manuscript preparation. P.L. Kumar, M-L.N. McDonald and C.A. Vaz Fragoso contributed to the analysis on COPDGene. W. Kim, C. Laurie, B.A. Raby, J.C. Celedón, S. Won and M.H. Cho contributed to the critical revision of the manuscript. All authors contributed to the relevant sections and approved the final manuscript.

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Conflict of Interest Statements

Asthma Management Program (CAMP), and 2) 2691 African American current or former smokers from the COPDGene study.

In the 894 Costa Rican trios, we identified a genome-wide significant association ($p=2.16\times10^{-9}$) between exertional dyspnoea and the single nucleotide polymorphism (SNP) rs10165869, located on chromosome 2q37.3, that was replicated in the CAMP cohort (p=0.023) with the same direction of association (combined $p=3.28\times10^{-10}$). This association was not found in the African American participants from COPDGene. We also found suggestive evidence for an association between SNP rs10165869 and the atypical chemokine receptor 3 (ACKR3).

Our finding encourages the secondary association analysis of a wider range of phenotypes that characterise respiratory symptoms in other airway diseases/studies.

## Tweetable Abstract

WGS data from a family-based association study suggest that the replicated SNP variant rs10165869 is associated with exertional dyspnoea, likely through the expression of ACKR3 https://bit.ly/3a5ddBd

### Keywords

childhood asthma; whole-genome sequencing; family-based genome-wide association study; exertional dyspnea; *ACKR3*

## INTRODUCTION

Asthma is the most common chronic respiratory disease of childhood and is characterised by inflammation of the airways, leading to airflow obstruction, and increased airway responsiveness that is generally caused by innate and adaptive immune responses to inhaled irritants and/or allergens [1]. The respiratory symptoms of an individual diagnosed with asthma consist of cough, dyspnoea, phlegm, wheezing and chest tightness, all of which are important markers of overall respiratory function and can signal asthma exacerbation [2].

Environmental exposures such as second-hand or personal exposure to tobacco smoke, viral respiratory infections, air pollutants and allergens can contribute to these respiratory symptoms, but do not account for all of them. Reporting or perception of respiratory symptoms differs among children with similar peak expiratory flow rate or forced expiratory volume in 1 s (FEV1), and such perceptual accuracy of lung function has been shown to further vary by ethnicity [3]. Moreover, spirometry is usually measured at rest and therefore does not explain exertional symptoms (e.g. dyspnoea) due to expiratory flow limitation and dynamic hyperinflation in response to exercise [4]. These data suggest that genetic variants may influence respiratory symptoms in children and that the magnitude of the effect of such variants may differ across ethnic groups.

To date, genome-wide association studies (GWAS) investigating genetic susceptibility to asthma have been limited to asthma affection status, lung function measures, asthma severity and response to asthma medications [5, 6]. Thus, GWAS of the respiratory symptoms are

rare, particularly in children. While there have been some studies of mucus hypersecretion in adult smokers and of respiratory symptoms in general population-based cohorts, no such studies have been conducted in individuals with asthma [7, 8].

In this study, we performed a genome-wide association analysis using whole-genome sequencing (WGS) data for five major respiratory symptoms in a family-based study of childhood asthma in Costa Rica. We then attempted to replicate our findings in a WGS family-based multi-ethnic study of childhood asthma in North America. Because childhood asthma has been associated with the development of chronic obstructive pulmonary disease (COPD), and the prevalence of childhood asthma is increased in African Americans [9], we further attempted to replicate our findings among African Americans in the COPDGene study.

## METHODS

### GWAS Subjects

Subject recruitment and the study protocol for the Genetic Epidemiology of Asthma in Costa Rica Study (GACRS) have been described in detail previously [10, 11]. In brief, GACRS is a family-based study of children with asthma. All participants were 6–14 years old and had asthma diagnosed by a physician and at least two respiratory symptoms (wheezing, cough or dyspnoea) or a history of asthma attacks in the previous year [12]. All participants also had at least six great-grandparents born in the Central Valley of Costa Rica, to ensure their descent from a founder population predominantly composed of Spaniards and Amerindians. The population is considered to be a semi-genetic isolate with Spanish and Amerindian ancestry owing to topographical separation from the coasts and countries to the north and south by mountain ranges.

We first attempted to replicate our findings in asthmatic offspring trios from the Childhood Asthma Management Program (CAMP). CAMP was a multicentre clinical trial designed to determine the long-term effects of three inhaled treatments for childhood asthma [13]. All of the participants had to have increased airway responsiveness (a provocative concentration causing a 20% fall in FEV1 $\leqslant$12.5 mg·mL$^{-1}$ of methacholine) and at least one of the following for at least 6 months in the previous year: respiratory symptoms at least twice per week, use of an inhaled bronchodilator at least twice per week or daily medication use for asthma. Children with severe asthma or other chronic medical conditions were excluded. Compared with GACRS, more participants in CAMP had moderate persistent asthma. We also attempted to replicate our results in African American current or former adult smokers in COPDGene. Subject recruitment and the study protocol for COPDGene have been described in detail previously [14].

Written parental consent and/or the subject's assent were obtained for each study protocol and ancillary genetic testing. Study protocols were approved by local Institutional Review Boards at each recruitment site for both studies, and by the Institutional Review Board of Brigham and Women's Hospital.

## WGS data

WGS data for GACRS, CAMP and COPDGene were generated as part of the National Heart, Lung, and Blood Institute (NHLBI) Trans-Omics for Precision Medicine (TOPMed) programme [15]. Further information can be found in the supplementary material. After confirming pedigree information and removing duplicates based on genome-wide identity-by-descent estimates, generated by kinship-based inference for GWAS (KING) [16], WGS data for 894 GACRS trios (a total of 2682 subjects), 286 CAMP trios (216 European American, 28 African American, 19 Latinx, 23 other, with a total of 858 subjects) and 2691 COPDGene African American subjects were obtained. For variant quality control, single nucleotide polymorphisms (SNPs) with Mendelian errors (>3), genotype missing rate (⩾2%), minor allele frequency (<1%) or deviations from Hardy-Weinberg proportions ($p<10^{-8}$) were removed.

## Respiratory Symptoms

According to the standard practice in epidemiology studies of children between the ages of 6 and 14, questionnaires were used to obtain data on respiratory symptoms in GACRS, including usual cough, usual phlegm, wheezing without cold, exertional dyspnoea and exertional chest tightness. The questionnaires were administered at the same time as spirometry measurement or collection of blood samples. For each respiratory symptom, the child or her/his parent (usually the mother) had to answer “Yes” to the following questions: “Does the child usually have cough when he/she does not have a cold or a flu?”, “Is the child usually congested or does he/she bring up phlegm when he/she does not have a cold or a flu?”, “Has the child’s chest ever sounded wheezy or whistling when he/she did not have a cold or a flu?”, “Has there ever been an occasion in the child’s life when he/she had an attack of shortness of breath when hurrying on the level or walking up a slight hill?” and “Has there ever been an occasion in the child’s life when he/she had tightness in his/her chest when hurrying on the level or walking up a slight hill?”

## Statistical analysis

Demographic characteristics and clinical features between groups depending on specific respiratory symptoms were compared using t-tests or Chi-squared tests, as appropriate. Association analysis in GACRS was conducted using the family-based association test (FBAT) software (version 2.04) with an additive genetic model and regression-based phenotype adjustment for age, sex, body mass index (BMI), lung function (FEV1/forced vital capacity (FVC)), $\log_{10}$(total IgE) and use of systemic oral corticosteroids in the previous year (as a proxy for asthma severity) [17]. For the top variants showing genome-wide significance, we also performed haplotype-based analysis using the same adjustment in GACRS. Because ~60% and 80% of the asthmatic subjects reported each respiratory symptom, the phenotype information for respiratory symptoms is similar to asthma affection status. To distinguish our associations from asthma-related associations, we investigated the transmission pattern in symptom-specific subgroups separately. We also analysed the association p-value for asthma affection status of the top significant variant. In an attempt to replicate the genome-wide significant hits, we performed FBAT analysis for respiratory

symptoms based on the CAMP trios at 2-year follow-up using the same covariate adjustment of the phenotypes.

In the COPDGene cohort, EPACTS (https://genome.sph.umich.edu/wiki/EPACTS) was used after adjusting for age, sex, Global Lung Function Initiative-calculated spirometric z scores [18], pack-years of smoking and top six principal components adjusting for population structure. We explored the link between the genetic variants and potential candidate genes using Open Targets Genetics (https://genetics.opentargets.org) and the cis-expression quantitative trait loci (eQTL) in lung tissue based on the GTEx release version 8 database (www.gtexportal.org/home). Topologically associated domains in lung tissue were also checked in a three-dimensional genome browser (Hi-C Unifying Genomic Interrogator, https://yunliweb.its.unc.edu/hugin). R statistical software (www.R-project.org) was used to evaluate these tests.

## RESULTS

### Descriptive characteristics of children with asthma

The characteristics of the 894 GACRS children with asthma are summarised in table 1. The median age of study participants was 9.1 years with a sex ratio of 1:0.7 (male:female) and a median age of asthma onset of 2.0 years (range=0–12 years). Most children (83.6%) had at least one positive skin test to allergens and their median FEV1 % pred was 97.5%. In CAMP, study participants had a median age of 8.8 years with a sex ratio of 1:0.5 (male:female) and a median age of asthma onset of 2.0 years (range=0–11 years) (table 1).

Table 2 shows the main characteristics of participants in GACRS according to the presence of each respiratory symptom. Subjects with cough or phlegm were slightly shorter than those without these symptoms, and children with exertional dyspnoea or chest tightness had a higher BMI than those without such symptoms. As expected, airway hyperresponsiveness and bronchodilator response were more common in children with wheezing, exertional dyspnoea or chest tightness than in those without these symptoms. Children with wheezing had a higher rate of positive skin prick tests than those without wheezing, and children with phlegm showed slightly higher eosinophil counts than those without phlegm. Subjects with each respiratory symptom were more likely to receive systemic corticosteroid medication compared to those without symptoms.

### GWAS of respiratory symptoms

Genome-wide FBAT results are displayed in quantile–quantile and Manhattan plots (figure 1). In this analysis, SNP rs10165869 (located on chromosome 2q37.3) was associated with exertional dyspnoea ($p$=2.16×10$^{-9}$). This association remained significant even after a Bonferroni correction for the five phenotypes tested (i.e. p<1.0×10$^{-8}$ or 5×10$^{-8}$ divided by 5). SNPs near rs10165869, including rs6725280, rs1865671, rs7607911, rs30102 and rs10168628, had $p$-values that achieved genome-wide significance, ranging from 3.77×10$^{-9}$ to 3.97×10$^{-8}$ (figure 1d). A haplotype-based test using all six SNPs identified a haplotype that is significantly associated with exertional dyspnoea ($p$=1.30×10$^{-8}$, supplementary table S1, supplementary figure S1) [19].

Using asthma affection status as the target phenotype, SNP rs10165869 was not significant ($p$=0.247). Subgroup analyses of the GACRS children either with or without exertional dyspnoea disclosed different transmission behaviour of the minor allele in each group (z=3.744 overtransmission, $p=1.81\times10^{-4}$, number of informative families=447 versus z= −4.704 undertransmission, $p=2.55\times10^{-6}$, number of informative families=120), which demonstrates the association between rs10165869 and exertional dyspnoea.

The SNP rs10165869 was replicated for exertional dyspnoea in the ethnically diverse CAMP study ($p$=0.023). The combined FBAT $p$-value was $3.28\times10^{-10}$. The corresponding subgroup analysis in CAMP showed the same pattern as in GACRS (z=1.353, $p$=0.176, number of informative families=102 versus z= −1.844, $p$=0.065, number of informative families=71). A subgroup analysis by ethnicity was also performed (supplementary table S2). rs10165869 was marginally associated with exertional dyspnoea in the African American subjects from COPDGene cohort but with the opposite effect direction ($p$=0.070).

### SNP rs10165869 and potential gene atypical chemokine receptor 3

According to the Open Targets Genetics database, the potential genes functionally implicated by rs10165869 are atypical chemokine receptor 3 (*ACKR3*), *COPS8*, *IQCA1* and *COL6A3*, from highest to lowest. The Hi-C Unifying Genomic Interrogator also captured a significant association between *ACKR3* and rs10165869 in lung tissue after Bonferroni correction ($p<1.0\times10^{-11}$, supplementary figure S2). According to the GTEx database, our top SNPs including rs10165869 are associated with an increased expression of *ACKR3* in lung tissue (supplementary table S3).

## Discussion

Based on our literature review, this is the first family-based association study of the five major respiratory symptoms in childhood asthma using WGS data. In this analysis, we identified rs10165869 as a novel SNP for exertional dyspnoea among Costa Rican children with asthma, with replication in the independent and ethnically diverse CAMP study. The dose-response slope to methacholine did not show any association in GACRS with the locus after the same adjustment ($p$=0.380), even though it was associated with both exertional phenotypes at baseline (table 2). Given that dyspnoea in childhood asthma is influenced by sex [20], we performed a sex interaction analysis on SNP rs10165869 using the unadjusted exertional dyspnoea phenotype but it was nonsignificant ($p$=0.379) [21].

ACKR3, also known as C-X-C chemokine receptor type 7 (CXCR7), is a G protein-coupled receptor (GPCR) for CXCL12, a chemokine that is involved in the inflammatory process regulating leukocyte extravasation into inflamed tissues [22, 23]. In asthmatic subjects, CXCL12 has been found in high concentrations in bronchoalveolar lavage fluid, correlated with circulating leukocytes, thus suggesting a role in airway inflammation and airway hyperresponsiveness [22]. CXCR4 had long been investigated as the only GPCR for CXCL12; however, it has now been shown that ACKR3 has ~10-fold higher binding affinity for CXCL12 [23]. In a murine model with the characteristic features of asthma, knockdown of *ACKR3* by a lentiviral delivery system in the lung reduces mucus secretion, allergic airway inflammation, serum allergen-specific IgE production, T-cell cytokine production and

airway hyperresponsiveness [24]. The predominant role of ACKR3 in the CXCL12/CXCR4/ACKR3 axis contributing to airway inflammation has also been shown in the pulmonary epithelium, polymorphonuclear neutrophils (PMN) and transepithelial PMN migration [25].

Aside from inflammatory conditions, *CXCL12* and *ACKR3* are also highly expressed under hypoxic conditions in pro-angiogenic environments such as various tumours, where CXCL12 enhances angiogenesis through ACKR3 activation [22, 26]. *ACKR3* expression is more sensitive to hypoxia for up to 48 h compared to *CXCR4*, especially in lung endothelial cells (supplementary figure S3) [27]. Endothelial cell dysfunction in pathogenesis regeneration under alveolar hypoxia, similar to that found in lung disease, is chiefly mediated by ACKR3 [28]. The role of ACKR3 on pulmonary epithelial and endothelial cells in preclinical studies supports the idea that the rs10165869 variant affects the upregulation of *ACKR3* in the lung, resulting in more severe dyspnoea symptoms than seen in asthma patients with the wild type, in whom exertional dyspnoea is purely a hypoxic condition due to chronic asthma. Previous GWAS also suggested an important role for rs7607316 and rs144060362 near the *ACKR3* region as genetic risk factors for airflow obstruction related to COPD ($p$=3×10$^{-6}$, 2×10$^{-6}$ respectively) [29, 30]. Our finding was not replicated in COPDGene.

Our study has several limitations. First, answers to questions about respiratory symptoms are subjective and thus influenced by both the perception of symptoms and recall bias. However, the misclassification seeming non-differential may lead to null or weaker associations. Moreover, these concerns are ameliorated by replication of our results for exertional dyspnoea in an independent multi-ethnic cohort of children living in a different geographic location. Second, this is a cross-sectional analysis. Although genotypes do not vary over time, we were unable to assess the temporal stability or variable severity of the reported symptoms in children with asthma. Third, we lack data on the use of controller medications for asthma in GACRS, which would affect respiratory symptoms. Despite these challenges, our analysis highlights the significance of symptom-based GWAS to identify the genetic determinants of respiratory symptoms.

In conclusion, our study identified that SNP rs10165869 is associated with exertional dyspnoea among children with asthma, enabling a better understanding of exertional dyspnoea. We hope that our finding motivates the association analysis of a wider range of phenotypes that characterise respiratory symptoms in other airway diseases/studies.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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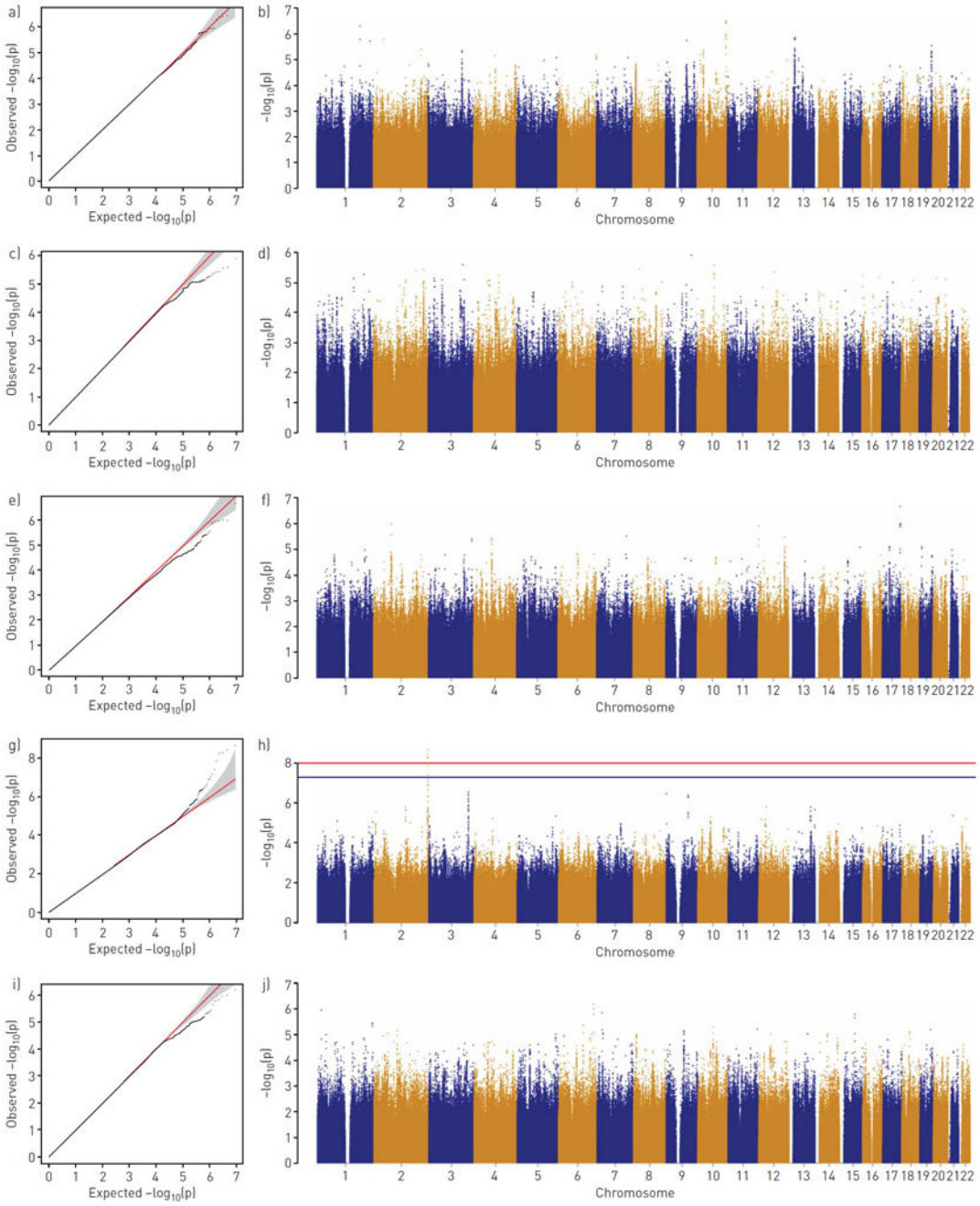


**Figure 1.**

Genome-wide association study based on 894 asthmatic Genetic Epidemiology of Asthma in Costa Rica Study trios for respiratory symptoms. a, b) Cough; c, d) phlegm; e, f) wheezing without cold; g, h) exertional dyspnoea; i, j) exertional chest tightness. Data visualised as quantile–quantile plots (a, c, e, g, i) and Manhattan plots (b, d, f, h, j). The red line corresponds to a Bonferroni-corrected genome-wide significance threshold for a total of five

phenotypes ($p$=1.0×10$^{-8}$) and the blue line indicates the commonly used genome-wide significance level ($p$=5×10$^{-8}$).

## TABLE 1

Demographic characteristics and clinical features of GACRS and CAMP subjects

| | GACRS Study | CAMP study | p-value |
|---|---|---|---|
| Subjects n | 894 | 286 | |
| Age years | 9.28 ± 1.86 | 8.90 ± 2.16 | $7.59\times10^{-3}$ |
| Male sex | 528 (59.06) | 193 (67.48) | 0.0134 |
| BMI $kg/m^2$ | 18.45 ± 3.92 | 18.24 ± 3.28 | 0.357 |
| Spirometric measures | | | |
| $FEV_1$ L | 1.80 ± 0.52 | 1.65 ± 0.47 | $1.03\times10^{-5}$ |
| $FEV_1$ % pred | 98.87 ± 16.84 | 92.82 ± 13.40 | $1.17\times10^{-9}$ |
| $FEV_1$/FVC % | 84.36 ± 7.79 | 79.69 ± 8.07 | $2.20\times10^{-16}$ |
| $FEV_1$/FVC % pred | 94.87 ± 8.70 | 90.36 ± 8.94 | $5.36\times10^{-13}$ |
| Bronchodilator Response as % of baseline $FEV_1$ | 5.58 ± 10.20 | 10.86 ± 9.49 | $7.50\times10^{-15}$ |
| $Log_{10}$ dose-response slope from saline | 1.15 ± 0.54 | 0.97 ± 0.47 | $1.92\times10^{-7}$ |
| Blood tests | | | |
| Total serum IgE $IU\cdot mL^{-1}$ | 725.26 ± 898.96 | 444.56 ± 797.47 | $7.89\times10^{-7}$ |
| Eosinophil, $count\cdot mm^{-2}$ | 553.84 ± 403.93 | 511.96 ± 425.43 | 0.148 |
| Positive skin tests to allergens | 3.08 ± 1.82 | 5.56 ± 4.20 | $2.20\times10^{-16}$ |

Data are presented as mean±SD or n (%), unless otherwise indicated. GACRS: Genetic Epidemiology of Asthma in Costa Rica Study; CAMP: Childhood Asthma Management Program; BMI: body mass index; $FEV_1$: forced expiratory volume in 1 s; FVC: forced vital capacity.

**TABLE 2**

Demographic characteristics and clinical features for different groups according to respiratory symptoms among the asthmatic GACRS children

| | Cough | | | Phlegm | | | Wheezing | | | Exertional Dyspnea | | | Exertional Chest tightness | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | Yes | p-value | No | Yes | p-value | No | Yes | p-value | No | Yes | p-value | No | Yes | p-value |
| Subjects | 190 (21.25) | 704 (78.75) | | 414 (46.31) | 479 (53.58) | | 105 (11.74) | 789 (88.26) | | 193 (21.59) | 701 (78.41) | | 266 (29.75) | 624 (69.80) | |
| Age years | 9.49 ± 1.84 | 9.22 ± 1.86 | 0.0773 | 9.52 ± 1.87 | 9.07 ± (1.83) | $3.07\times10^{-4}$ | 9.28 ± 1.84 | 9.28 ± 1.86 | 0.972 | 9.19 ± 1.94 | 9.30 ± 1.84 | 0.466 | 9.08 ± 1.77 | 9.35 ± 1.89 | 0.0428 |
| Male sex | 112 (58.95) | 416 (59.09) | 1 | 247 (59.66) | 281 (58.66) | 0.815 | 61 (58.10) | 467 (59.19) | 0.914 | 124 (64.25) | 404 (57.63) | 0.116 | 157 (59.02) | 369 (59.13) | 1 |
| Height m | 1.35 ± 0.12 | 1.32 ± (0.12) | $1.84\times10^{-3}$ | 1.35 ± 0.12 | 1.32 ± 0.12 | $5.13\times10^{-4}$ | 1.32 ± 0.12 | 1.33 ± 0.12 | 0.563 | 1.32 ± 0.13 | 1.33 ± 0.12 | 0.301 | 1.32 ± 0.12 | 1.33 ± 0.12 | 0.139 |
| BMI $kg/m^2$ | 18.67 ± 4.28 | 18.39 ± 3.81 | 0.413 | 18.75 ± 4.08 | 18.20 ± 3.76 | 0.0347 | 18.34 ± 3.85 | 18.47 ± 3.93 | 0.756 | 17.86 ± 3.54 | 18.62 ± 4.00 | 0.0114 | 18.01 ± 3.98 | 18.63 ± 3.89 | 0.0335 |
| Number of older siblings | 1.33 ± 1.43 | 1.09 ± 1.27 | 0.0366 | 1.16 ± 1.28 | 1.12 ± 1.33 | 0.656 | 1.11 ± 1.35 | 1.14 ± 1.31 | 0.784 | 1.15 ± 1.27 | 1.14 ± 1.32 | 0.912 | 1.11 ± 1.30 | 1.15 ± 1.32 | 0.640 |
| Spirometric measures | | | | | | | | | | | | | | | |
| FEV1/FVC % | 85.17 ± 7.53 | 84.14 ± 7.85 | 0.0967 | 84.56 ± 8.25 | 84.20 ± 7.37 | 0.495 | 84.80 ± 6.72 | 84.30 ± 7.92 | 0.492 | 84.91 ± 7.68 | 84.21 ± 7.82 | 0.266 | 84.89 ± 7.73 | 84.10 ± 7.81 | 0.161 |
| FEV1/FVC % pred | 95.84 ± 8.49 | 94.61 ± 8.74 | 0.0805 | 95.17 ± 9.29 | 94.64 ± 8.16 | 0.369 | 95.33 ± 7.72 | 94.81 ± 8.82 | 0.525 | 95.57 ± 8.51 | 94.68 ± 8.75 | 0.204 | 95.39 ± 8.60 | 94.60 ± 8.74 | 0.212 |
| Absolute response to bronchodilator mL | 95.33 ± 124.61 | 82.61 ± 139.98 | 0.230 | 83.28 ± 143.08 | 86.84 ± 131.34 | 0.703 | 63.52 ± 101.68 | 88.12 ± 140.53 | 0.0325 | 74.24 ± 125.28 | 88.41 ± 139.85 | 0.180 | 73.39 ± 133.75 | 90.39 ± 138.37 | 0.0897 |
| Dose-response slope to methacholine µmol | 28.94 ± 36.59 | 27.21 ± 40.58 | 0.603 | 28.10 ± 39.11 | 27.17 ± 40.37 | 0.747 | 22.23 ± 31.92 | 28.35 ± 40.74 | 0.0901 | 22.17 ± 33.16 | 29.03 ± 41.27 | 0.0267 | 21.44 ± 28.64 | 30.30 ± 43.54 | $9.08\times10^{-4}$ |
| $PD_{20}$ mg | 1.69 ± 2.21 | 1.84 ± 2.35 | 0.504 | 1.69 ± 2.36 | 1.91 ± 2.30 | 0.276 | 2.43 ± 2.86 | 1.71 ± 2.22 | 0.0379 | 2.20 ± 2.79 | 1.71 ± 2.18 | 0.0747 | 2.18 ± 2.63 | 1.67 ± 2.18 | 0.0298 |
| Blood tests | | | | | | | | | | | | | | | |
| Total serum IgE $IU{\cdot}mL^{-1}$ | 731.46 ± 982.71 | 723.59 ± 875.80 | 0.921 | 728.68 ± (927.43) | 713.37 ± 853.38 | 0.799 | 583.52 ± 863.08 | 744.19 ± 902.50 | 0.0770 | 674.34 ± 793.46 | 739.24 ± 925.88 | 0.334 | 676.38 ± 797.89 | 749.62 ± 940.18 | 0.237 |
| Positive IgE to dust mite | 132 (69.47) | 531 (75.43) | 0.103 | 303 (73.19) | 359 (74.95) | 0.606 | 71 (67.62) | 592 (75.03) | 0.120 | 141 (73.06) | 522 (74.47) | 0.716 | 194 (72.93) | 467 (74.84) | 0.556 |

| | Cough | | | Phlegm | | | Wheezing | | | Exertional Dyspnea | | | Exertional Chest tightness | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No** | **Yes** | **p-value** | **No** | **Yes** | **p-value** | **No** | **Yes** | **p-value** | **No** | **Yes** | **p-value** | **No** | **Yes** | **p-value** |
| Positive IgE to cockroach | 80 (42.11) | 307 (43.61) | 0.750 | 179 (43.24) | 207 (43.21) | 1 | 35 (33.33) | 355 (44.61) | 0.0350 | 81 (41.97) | 306 (43.65) | 0.714 | 104 (39.10) | 282 (45.19) | 0.100 |
| Positive IgE to Ascaris | 67 (35.26) | 267 (37.93) | 0.571 | 155 (37.44) | 178 (37.16) | 0.962 | 29 (27.62) | 305 (38.66) | 0.0343 | 62 (32.12) | 272 (38.80) | 0.111 | 88 (33.08) | 244 (39.10) | 0.105 |
| Eosinophil count·mm$^{-2}$ | 503.54 ± 382.75 | 567.39 ± 408.66 | 0.0481 | 517.06 ± 374.41 | 582.96 ± 424.11 | 0.0151 | 495.11 ± 436.92 | 561.62 ± 399.01 | 0.147 | 529.81 ± 406.98 | 560.45 ± 403.14 | 0.361 | 537.91 ± 407.68 | 563.57 ± 402.16 | 0.394 |
| Positive skin tests to allergens | 3.01 ± 1.86 | 3.10 ± 1.81 | 0.554 | 3.12 ± 1.81 | 3.06 ± 1.83 | 0.623 | 2.57 ± 1.96 | 3.15 ± 1.79 | $5.40\times10^{-3}$ | 3.15 ± 1.74 | 3.06 ± 1.84 | 0.531 | 3.18 ± 1.85 | 3.04 ± 1.80 | 0.335 |
| Systemic corticosteroid use last year | 135 (71.05) | 567 (80.54) | $6.41\times10^{-3}$ | 308 (74.40) | 393 (82.05) | $7.08\times10^{-3}$ | 67 (63.81) | 635 (80.48) | $1.56\times10^{-4}$ | 137 (70.98) | 565 (80.60) | $5.42\times10^{-3}$ | 193 (72.56) | 505 (80.93) | $7.13\times10^{-3}$ |

Data are presented as mean±SD or n (%), unless otherwise indicated. GACRS: Genetic Epidemiology of Asthma in Costa Rica Study; BMI: body mass index; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; PD20: the cumulative dose of methacholine required to produce a 20% fall in FEV1 from the post saline FEV1.