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## Authors

Li, Xingnan
Li, Huashi
Christenson, Stephanie
et al.

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# Genetic analyses of chr11p15.5 region identify *MUC5AC-MUC5B* associated with asthma-related phenotypes

**Xingnan Li, PhD**[1], **Huashi Li, MS**[1], **Stephanie A. Christenson, MD**[2], **Mario Castro, MD**[3], **Loren C. Denlinger, MD**[4], **Serpil C. Erzurum, MD**[5], **John V. Fahy, MD**[2], **Benjamin M. Gaston, MD**[6], **Elliot Israel, MD**[7], **Nizar N. Jarjour, MD**[4], **Bruce D. Levy, MD**[7], **David T Mauger, PhD**[8], **Wendy C. Moore, MD**[9], **Joe Zein, MD**[5], **Naftali Kaminski, MD**[10], **Sally E. Wenzel, MD**[11], **Prescott G. Woodruff, MD**[2], **Eugene R. Bleecker, MD**[1], **Deborah A. Meyers, PhD**[1], **NHLBI Severe Asthma Research Program (SARP)**

[1]Division of Genetics, Genomics and Precision Medicine, Department of Medicine, University of Arizona, Tucson, Arizona, USA

[2]Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of California at San Francisco, San Francisco, California, USA

**Correspondence:** Xingnan Li, PhD, MS, Division of Genetics, Genomics and Precision Medicine, Department of Medicine, University of Arizona College of Medicine, 1230 N Cherry Avenue, PO Box 210242, Tucson, AZ 85719, USA, Telephone: 520-626-2905, Fax: 520-626-1894, lixingnan1@deptofmed.arizona.edu or lixingnan1@yahoo.com.

Declaration of interest

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Supplemental online material

Additional supplemental online material may be found in the online version of this article.

[3]Division of Pulmonary, Critical Care and Sleep Medicine, University of Kansas School of Medicine, Kansas City, Kansas, USA

[4]Department of Medicine, University of Wisconsin School of Medicine & Public Health, Madison, Wisconsin, USA

[5]Lerner Research Institute and the Respiratory Institute, Cleveland Clinic, Cleveland, Ohio, USA

[6]Wells Center for Pediatric Research and Riley Hospital for Children, Indiana University, Indianapolis, Indiana, USA

[7]Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA

[8]Department of Public Health Sciences, College of Medicine, Penn State University, Hershey, Pennsylvania, USA

[9]Department of Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA

[10]Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, Yale School of Medicine, New Haven, Connecticut, USA

[11]Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

## Abstract

**Objective:** Genome-wide association studies (GWASs) have identified single nucleotide polymorphisms (SNPs) in chr11p15.5 region associated with asthma and idiopathic interstitial pneumonias (IIPs). We sought to identify functional genes for asthma by combining SNPs and mRNA expression in bronchial epithelial cells (BEC) in the Severe Asthma Research Program (SARP).

**Methods:** Correlation analyses of mRNA expression of six candidate genes (*AP2A2*, *MUC6*, *MUC2*, *MUC5AC*, *MUC5B*, and *TOLLIP*) and asthma phenotypes were performed in the longitudinal cohort (n = 156) with RNAseq in BEC, and replicated in the cross-sectional cohort (n = 155). eQTL (n = 114) and genetic association analysis of asthma severity (426 severe vs. 531 non-severe asthma) were performed, and compared with previously published GWASs of IIPs and asthma.

**Results:** Higher expression of *AP2A2* and *MUC5AC* and lower expression of *MUC5B* in BEC were correlated with asthma, asthma exacerbations, and T2 biomarkers ($P < 0.01$). SNPs associated with asthma and IIPs in previous GWASs were eQTL SNPs for *MUC5AC*, *MUC5B*, or *TOLLIP*, however, they were not in strong linkage disequilibrium. The risk alleles for asthma or protective alleles for IIPs were associated with higher expression of *MUC5AC* and lower expression of *MUC5B*. rs11603634, rs12788104, and rs28415845 associated with moderate-to-severe asthma or adult onset asthma in previous GWASs were not associated with asthma severity ($P > 0.8$).

**Conclusions:** SNPs associated with asthma in chr11p15.5 region are not associated with asthma severity neither with IIPs. Higher expression of *MUC5AC* and lower expression of *MUC5B* are risk for asthma but protective for IIPs.

**Keywords**

Asthma susceptibility; asthma severity; eQTL; gene expression; genetic association; *MUC5AC*; *MUC5B*

## Introduction

Airway mucus hypersecretion is associated with asthma and chronic obstructive pulmonary disease (COPD) [1–2]. Four secreted gel-forming mucin genes (*MUC6*, *MUC2*, *MUC5AC*, and *MUC5B*) are clustered in human chromosome 11p15.5 region [3–4]. *MUC5AC* and *MUC5B* are predominantly expressed mucin genes in the airway, mainly expressed in the tracheal and bronchial epithelium, and localized to goblet cells and submucosal gland, respectively [4]. In this study, we investigated mRNA expression of six candidate genes (*AP2A2*, *MUC6*, *MUC2*, *MUC5AC*, *MUC5B*, and *TOLLIP*) in chr11p15.5 region using mRNA expression data in bronchial epithelial cells (BEC) from the SARP cohorts to dissect the correlation of gene expression and asthma phenotypes.

rs7104956 in *AP2A2* has been associated with asthma in European Americans and African Americans ($P = 2.4 \times 10^{-7}$) [5]. More recently, rs11603634 in *MUC2-MUC5AC* region has been reported to be associated with severe asthma ($P = 2.3 \times 10^{-8}$; moderate-to-severe asthma vs. healthy control) [6]; rs28415845 ($P = 2.9 \times 10^{-13}$), rs12788104 ($P = 1.4 \times 10^{-8}$) and rs35225972 ($P = 4 \times 10^{-10}$) in *MUC2-MUC5AC* region has been associated with adult onset asthma [7–8] and asthma [9–11]. In addition, multiple SNPs in *MUC2-MUC5AC* region have been associated with asthma in a large meta-analysis of GWAS of asthma ($P < 10^{-5}$) [12]. The published GWASs have tested association of SNPs with asthma susceptibility (asthma vs. healthy control) or severe asthma (moderate-to-severe asthma vs. healthy control). In this study, we performed genetic association analysis of asthma severity (severe asthma vs. non-severe asthma) in chr11p15.5 region to investigate whether SNPs associated with asthma or severe asthma are also associated with asthma severity.

GWASs have also identified SNPs in *MUC2*, *MUC5AC*, *MUC5B*, and *TOLLIP* associated with idiopathic interstitial pneumonias (IIPs) and idiopathic pulmonary fibrosis (IPF) [13–19]. IIPs are heterogeneous interstitial pulmonary diseases characterized by interstitial inflammation and fibrosis with unknown etiology [20]. IPF is the most common subphenotype of IIPs. In this study, we comprehensively investigated all the SNPs identified through GWASs of asthma and IIPs in this region [21]. eQTL analysis was performed for these candidate SNPs in BEC and compared with published eQTL in the lung tissue from GTEx database [22] to dissect potential functional genes or SNPs for asthma and IIPs.

In order to delineate the functional genes for asthma in this region, we utilized a unique dataset of mRNA expression in BEC from patients with asthma. We hypothesize that combining SNP data and mRNA expression data, we will be able to determine the functional asthma genes in this complicated chromosomal region.

# Methods

## Study participants

The main goal of SARP is to characterize severe asthma in comparison to non-severe asthma. The earlier SARP cohort was a cross-sectional study [23–25] and the current SARP cohort is an ongoing longitudinal study [26–28]. Participants were extensively phenotyped using standardized procedures, which included asthma-related quality of life, spirometry, and sputum induction. The longitudinal cohort has three-year longitudinal data and the cohort is still being followed (www.severeasthma.org). Bronchoscopy was performed on a subset of SARP cohorts to obtain epithelial cells from brush biopsies for mRNA expression analysis. SARP studies were approved by institutional review boards, including informed consent from all participants.

## Statistical Analyses

Whole-genome sequence (WGS) and standard quality control (QC) process of sequencing data in SARP (dbGaP accession: phs001446) were performed through NHLBI-sponsored TOPMed Program (www.nhlbiwgs.org). SNPs were extracted (hg38: *AP2A2* to *TOLLIP*; chr11:925,881–1,309,622) using PLINK 1.9 software [29]. Further QC of participants and SNPs were performed as described before [30–31].

A set of 26 candidate SNPs was selected based on GWASs of asthma and IIPs ($P < 10^{-5}$) from NHGRI-EBI GWAS catalog (www.ebi.ac.uk/gwas/; accessed on August 18, 2022) [21] and related GWAS [12]. The SNPs associated with asthma ($P < 10^{-5}$) were extracted from a large meta-analysis of GWAS of asthma (TAGC study; EMBI-EBI access number: GCST006862) [12]. Linkage disequilibrium (LD) was estimated with 95% confidence intervals of D' to define LD blocks and LD plots of 26 candidate SNPs were generated for 1,016 non-Hispanic Whites in SARP using Haploview [32].

Logistic regression, assuming a genetic additive model, was used for genetic association analysis of asthma severity (426 severe asthma vs. 531 non-severe asthma) in SARP non-Hispanic White adults (age ≥ 12 years old), adjusted for age, sex, and the first five components from the multidimensional scaling analysis of genome as described [31]. Due to relative small sample size, only 26 candidate SNPs were tested and nominal p-value of 0.05 was considered as significant.

RNAseq data from human BEC brushing in the longitudinal cohort were extracted for five candidate genes (except for *MUC2*, which failed QC). In brief, Illumina HiSeq RNAseq reads were quality filtered and mapped to human genome hg38 using STAR package [33]. Read counts were regularized logarithm transformed using DESeq2 package [34]. The RNAseq data have been deposited (dbGaP accession: phs001446) [31,35]. Agilent Whole Human Genome Microarray 4×44K v2 expression data of six candidate genes were extracted from BEC in the cross-sectional cohort as described previously [31,36–37]. The microarray expression data have been deposited (GEO accession: GSE63142 and GSE43696).

Correlation analysis of mRNA expression levels and asthma-related phenotypes was performed as described [31]. In brief, a linear or logistic model was used to test correlation between mRNA expression levels of six genes and asthma-related phenotypes with adjustment of age, sex, race, BMI, and batch effect. The primary phenotype studied was asthma susceptibility, and other related phenotypes were also tested. The longitudinal cohort was used as discovery dataset, and the cross-sectional cohort was used as replication dataset. P-value less than 0.0083 (0.05/6 genes) was considered as significant level for asthma susceptibility. P-value less than 0.05 was considered as significant for other asthma-related phenotypes.

Gene expression correlation analysis between six candidate genes and five gene expression biomarkers (*POSTN* and *IL13* [Th2], *IL12A* [Th1], *IL17A* [Th17], *IL6* [inflammation]) in BEC in the longitudinal cohort was performed using Spearman rank test. P-value less than 0.0017 (0.05/30 tests) was used as significant level.

eQTL analysis was performed as described previously [31]. In brief, a linear additive genetic model was used to test association between SNPs and inverse normalized mRNA expression data in the longitudinal cohort with WGS and RNAseq in BEC (n = 114). Due to relative small sample size, eQTL analysis was only performed on 26 candidate SNPs and nominal p-value of 0.05 was considered as significant. Candidate SNPs were also checked for significant eQTLs in the lung tissue (n = 383) from Genotype-Tissue Expression (GTEx) database [22].

## Results

### Expression Analyses

Six candidate genes in chr11p15.5 region (Figure S1) were selected for investigation based on the published GWASs of asthma and IIPs [21]. Correlation analysis of mRNA expression levels and asthma phenotypes was performed in BEC in the longitudinal cohort (n = 156), and replicated in the cross-sectional cohort (n = 155) (Table S1).

In the longitudinal cohort, higher mRNA expression levels of *AP2A2* and *MUC5AC* or lower mRNA expression levels of *MUC5B* were significantly correlated with asthma susceptibility ($P < 0.05/6 = 0.008$) (Table 1 and Figure 1). At nominal p-value of 0.05, the higher mRNA expression levels of *AP2A2* were correlated with asthma severity ($P = 0.05$) in the cross-sectional cohort and greater number of exacerbations due to asthma in the last 12 months ($P = 0.0008$) and in the prospective three years ($P = 0.03$) in the longitudinal cohort. The higher mRNA expression levels of *MUC5AC* were correlated with lower baseline % predicted $FEV_1$ in the longitudinal cohort ($P = 0.004$) and the cross-sectional cohort ($P = 0.03$), greater number of exacerbations in the last 12 months ($P = 0.03$) and in the prospective three years ($P = 0.007$) in the longitudinal cohort, and greater percentage of emergency room (ER) visit or hospitalization due to asthma in the last 12 months in the cross-sectional cohort ($P = 0.009$). The lower mRNA expression levels of *MUC5B* were correlated with asthma susceptibility in the cross-sectional cohort ($P = 0.0003$), lower baseline % predicted $FEV_1$ ($P = 0.009$), and greater number of exacerbations in the last 12 months in the longitudinal cohort ($P = 0.004$).

In addition, the higher mRNA expression levels of *AP2A2* and *MU5AC* or lower mRNA expression levels of *MUC5B* were significantly correlated with T2 biomarkers ($P < 0.05$), such as blood and sputum eosinophils, fractional exhaled nitric oxide (FeNO), total serum IgE levels in the longitudinal cohort (Table 2 and Figure 2), and these findings were replicated in the similar trend in the cross-sectional cohort. Furthermore, the ratio of mRNA expression levels of *MUC5AC* to *MUC5B* was significantly correlated with asthma phenotypes (Table 1–2). The mRNA expression levels of *MUC2*, *MUC6*, and *TOLLIP* were either not significantly or not consistently correlated with asthma phenotypes. These results indicated that the higher mRNA expression levels of *AP2A2* and *MUC5AC* or the lower mRNA expression levels of *MUC5B* were correlated with asthma phenotypes.

Gene expression correlation between six candidate genes and five biomarkers (*POSTN* and *IL13* [Th2], *IL12A* [Th1], *IL17A* [Th17], and *IL6* [inflammation]) was tested (Table S2). The mRNA expression levels of *MUC5AC* were positively correlated with *POSTN* and negatively correlated with *IL12A* and *IL6*. The mRNA expression levels of *MUC5B* were positively correlated with *IL12A* and negatively correlated with *IL13* and *POSTN*. These findings indicated that *MUC5AC* and *MUC5B* might be involved in Th2 and Th1 pathways, respectively.

## Comparison of GWASs and eQTL

To investigate gene expression regulation in this region, eQTL analysis was performed in BEC in the longitudinal cohort (n = 114; Table S1) and the results were compared with public eQTL database of GTEx [22], and GWASs of asthma and IIPs [12,21] (Table 3). LD analysis of 26 GWAS-identified candidate SNPs in 1,016 non-Hispanic Whites in SARP identified four LD blocks (Figure S2).

rs7104956 in *AP2A2* has been associated with asthma [5] and the risk allele C for asthma was associated with higher mRNA expression of *MUC5AC* in SARP ($P = 0.03$). Two SNPs (rs7934606 and rs6421972) in *MUC2* (LD block 1) have been associated with IIPs [13–14], however, they were not associated with asthma in previous GWASs neither with mRNA expression levels of six candidate genes in this study.

11 SNPs in *MUC2-MUC5AC* region (LD block 2) have been associated with asthma [6,8–12] and the risk alleles for asthma were associated with higher mRNA expression of *MUC5AC* and/or lower mRNA expression of *MUC5B*. For example, rs11603634 has been associated with severe asthma ($P = 2.3 \times 10^{-8}$; moderate-to-severe asthma vs. healthy control) [6]. G allele of rs11603634 was the risk allele for severe asthma and associated with higher mRNA expression of *MUC5AC* in BEC ($P = 2.5 \times 10^{-5}$) [6] and in BEC in SARP ($P = 0.006$). Two SNPs (rs12788104 and rs28415845) in *MUC2-MUC5AC* region have been associated with adult onset asthma [7–8] and the risk alleles for adult onset asthma were associated with higher mRNA expression of *MUC5AC* and/or lower mRNA expression of *MUC5B* in SARP. Importantly, these three SNPs (rs11603634, rs12788104, and rs28415845) were not associated with asthma severity ($0.98 \leq$ Odds ratio $< 1$, $P > 0.8$; severe asthma vs. non-severe asthma) in SARP. In conclusion, the SNPs in *MUC2-MUC5AC* (LD block 2) region were associated with asthma but not associated with asthma severity nor IIPs in previous GWASs.

Two SNPs (rs28403537 and rs35288961) in *MUC5AC* associated with IIPs [14] were not associated with asthma severity in SARP. T allele of rs35288961 was the risk allele for IIPs [14] and associated with lower mRNA expression of *TOLLIP* in SARP ($P = 0.04$). Three SNPs (rs34595903, rs2672794, and rs35705950) in *MUC5B* (LD block 3) were associated with IIPs [13–19] and the risk alleles for IIPs were associated with higher mRNA expression of *MUC5B* and/or lower mRNA expression of *MUC5AC* in SARP. Four SNPs in *TOLLIP* (LD block 4) were associated with IIPs [13,16] and the risk alleles for IIPs (except rs5743890) were associated with lower mRNA expression of *TOLLIP* in the lung tissue [16]. T allele of rs5743890 was the risk allele for IIPs [16], however, it was associated with higher mRNA expression of *TOLLIP* in SARP ($P = 0.004$). In conclusion, the SNPs in *MUC5AC*, *MUC5B*, and *TOLLIP* (LD blocks 3 and 4) associated with IIPs were not associated with asthma or asthma severity.

In summary, SNPs associated with asthma or IIPs are eQTL SNPs for *MUC5AC*, *MUC5B*, or *TOLLIP*. SNPs associated with asthma were not associated with asthma severity. SNPs associated with asthma and IIPs were not overlapped nor in strong LD. Higher mRNA expression of *MUC5AC* and lower mRNA expression of *MUC5B* are risk for asthma but protective for IIPs.

## Discussion

In this study, we comprehensively investigated the mRNA expression of six genes in chr11p15.5 region. Expression analyses indicated that higher mRNA expression levels of *AP2A2* and *MUC5AC* or lower mRNA expression levels of *MUC5B* were significantly and consistently correlated with asthma phenotypes in BEC. In addition, the ratio of mRNA expression levels of *MUC5AC* to *MUC5B* was significantly correlated with asthma phenotypes. Previous studies have indicated that the ratio of MUC5AC to MUC5B sputum protein levels is higher in patients with asthma [38] or acute asthma [39] compared with healthy controls. All these findings consistently indicate that higher expression of *MUC5AC* and lower expression of *MUC5B* or higher ratio of *MUC5AC* to *MUC5B* are associated with asthma and may be used as biomarkers for asthma.

The higher mRNA expression of *AP2A2* and *MUC5AC* or lower mRNA expression of *MUC5B* were correlated with T2 biomarkers such as blood and sputum eosinophils, FeNO, and total serum IgE levels. In addition, the mRNA expression levels of *MUC5AC* were positively correlated with *POSTN*, which encodes periostin (Th2 biomarker), and negatively correlated with *IL12A* (Th1 biomarker) and *IL6* (neutrophilic inflammation biomarker). By contrast, the mRNA expression levels of *MUC5B* were positively correlated with *IL12A* and negatively correlated with *IL13* and *POSTN*. In a previous gene expression study, patients with Th2-high asthma have elevated *MUC5AC* and repressed *MUC5B* mRNA expression in BEC compared with healthy controls [40]. In a recent study, mucus plugs have been found to be correlated with airway obstruction and eosinophilia in subjects with severe asthma [41]. All these findings indicate that *MUC5AC* and *MUC5B* are involved in Th2 and Th1 pathways, respectively.

In this study, we found that SNPs associated with asthma were not associated with asthma severity. Shrine et al. performed a GWAS by comparing patients with moderate-to-severe asthma and non-allergic healthy controls and identified three novel genes (*MUC5AC*, *GATA3*, and *KIAA1109*) [6]. This type of comparison is more likely to identify the same set of genes associated with asthma susceptibility and/or allergy. Our findings indicate that SNPs in *MUC2-MUC5AC* region associated with asthma susceptibility are correlated with higher mRNA expression of *MUC5AC* and/or lower mRNA expression of *MUC5B*, however, these SNPs are not associated with asthma severity. Our previous study also indicated that Th2 pathway genes are associated with asthma susceptibility, however, Th1 pathway genes and lung function genes are associated with asthma severity [42]. We also identified that SNPs associated with asthma and IIPs were not overlapped nor in strong LD. Thus, although SNPs in chr11p15.5 region have been associated with asthma and IIPs, different sets of SNPs are associated with asthma or IIPs.

The gene expression patterns of *MUC5AC* and *MUC5B* are not consistent for different pulmonary diseases. In patients with cystic fibrosis, sputum MUC5AC and MUC5B protein levels are lower in mucus than healthy controls [43]. In patients with chronic rhinosinusitis, mRNA levels of *MUC5AC* and *MUC5B* are higher than healthy controls [44]. In patients with COPD, increased sputum MUC5AC protein levels are associated with COPD development, progression, and exacerbations [45]. A COPD study has indicated that sputum MUC5AC and MUC5B protein levels are higher in smokers than non-smokers and higher in subjects with chronic bronchitis than those without chronic bronchitis [46]. In this study, we showed that higher mRNA expression of *MUC5AC* and lower mRNA expression of *MUC5B* are risk for asthma but protective for IIPs and distinct sets of SNPs are associated with asthma or IIPs, which may indicate different gene expression regulatory mechanisms in asthma and IIPs.

MUC5B is the predominant mucin in the normal airway and required for mucociliary clearance to reduce inhaled particles and control infections in the airway [47–48]. The overexpression of MUC5B in IIPs may be due to a repair process for airway epithelial progenitors and may lead to mesenchymal cell proliferation and fibrosis [48]. MUC5AC is a “response” mucin that is inducible by allergens and viruses and required for allergic airway hyperresponsiveness [49]. MUC5AC enrichment in mucus may impair mucociliary transport by tethering airway epithelial cells and form mucus plugs in asthma [50]. In the pathogenesis of asthma, higher expression of *MUC5AC* may lead to airway hyperresponsiveness and mucus plugs and lower expression of *MUC5B* may weaken airway defense. These two processes may work together to worsen asthma symptoms.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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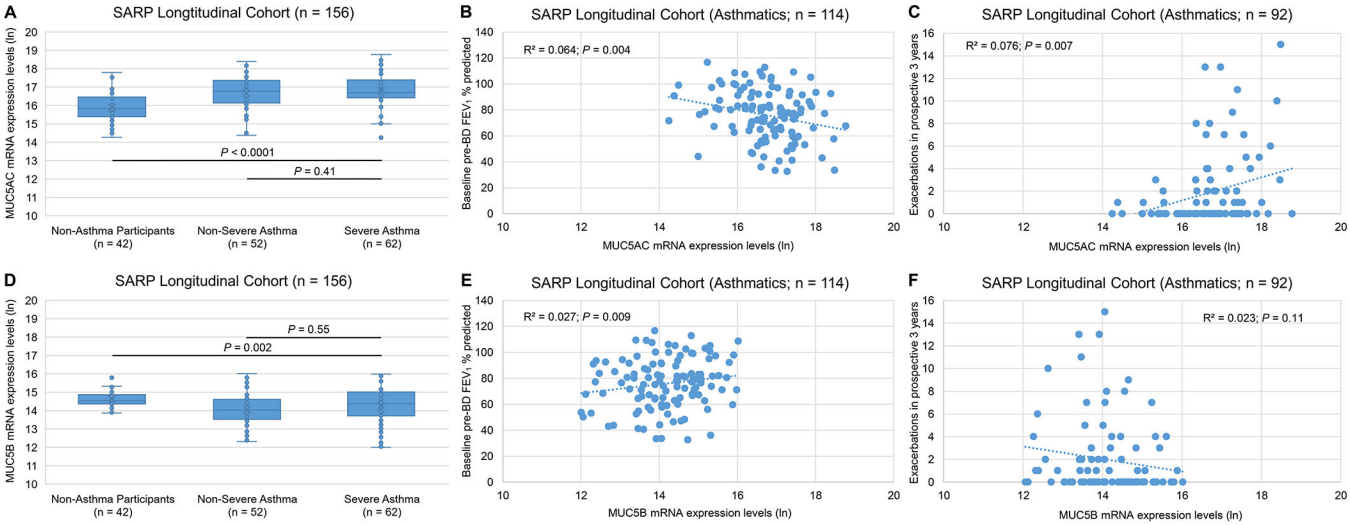


**Figure 1.**

Association of *MUC5AC* and *MUC5B* mRNA expression levels in bronchial epithelial cells with (A and D) asthma susceptibility and asthma severity, (B and E) baseline pre-BD $FEV_1$ % predicted, and (C and F) asthma exacerbations in prospective 3 years in the SARP (Severe Asthma Research Program) longitudinal cohort. *MUC5AC* and *MUC5B* mRNA expression levels have been natural logarithm (ln)-transformed in the (A-F) SARP longitudinal cohort. Pre-BD=pre-bronchodilator.

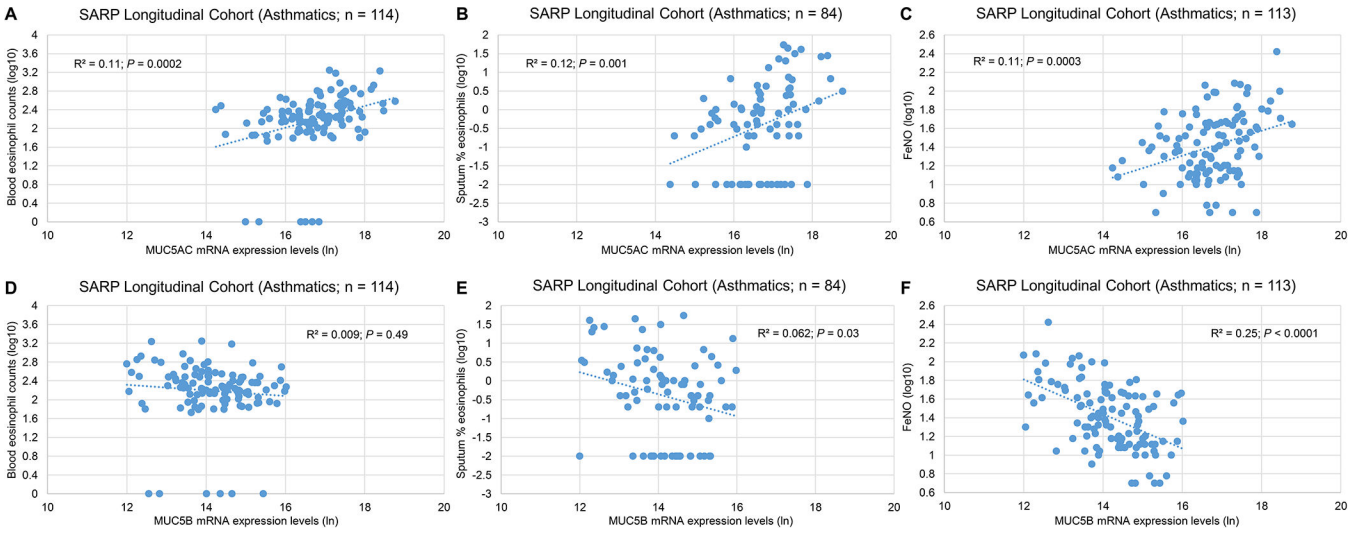


**Figure 2.**

Association of *MUC5AC* and *MUC5B* mRNA expression levels in bronchial epithelial cells with (A and D) blood eosinophil counts, (B and E) sputum percentage eosinophils, and (C and F) fractional exhaled nitric oxide (FeNO) values in the SARP (Severe Asthma Research Program) longitudinal cohort. *MUC5AC* and *MUC5B* mRNA expression levels have been natural logarithm (ln)-transformed in the (A-F) SARP longitudinal cohort.

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**Table 1.**

Correlation of mRNA expression levels of six candidate genes in chr11p15.5 region and asthma-related phenotypes in bronchial epithelial cells in SARP.

| **SARP Longitudinal Cohort RNA-Seq (n = 156)*** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gene** | **Asthma Susceptibility** | | | **Asthma Severity** | | | **Baseline Pre-BD $FEV_1$ % Predicted** | | **Number of Exacerbations (last 12 months)** | | **Number of Exacerbations (prospective 3 years)** | |
| | **Non-Asthma Participants (n = 42)** | **Patients with Asthma (n = 114)** | ***P* value†** | **Non-Severe Asthma (n = 52)** | **Severe Asthma (n = 62)** | ***P* value†** | **β‡** | ***P* value‡ (n = 114)** | **β‡** | **P value‡ (n = 114)** | **β‡** | ***P* value‡ (n = 92)** |
| *AP2A2* | 10.2 ± 0.14 | 10.3 ± 0.16 | 0.0023 | 10.3 ± 0.17 | 10.3 ± 0.14 | 0.20 | −17.41 | 0.12 | 5.85 | 0.0008 | 5.22 | 0.025 |
| *MUC6* | 2.12 ± 0.53 | 2.57 ± 1.16 | 0.0094 | 2.29 ± 0.89 | 2.81 ± 1.31 | 0.11 | 1.37 | 0.39 | −0.23 | 0.37 | 0.09 | 0.82 |
| *MUC5AC* | 15.9 ± 0.82 | 16.7 ± 0.87 | <0.0001 | 16.7 ± 0.90 | 16.8 ± 0.85 | 0.41 | −5.58 | 0.0035 | 0.67 | 0.03 | 1.07 | 0.0068 |
| *MUC5B* | 14.6 ± 0.47 | 14.2 ± 0.94 | 0.0017 | 14.1 ± 0.87 | 14.3 ± 0.99 | 0.55 | 4.75 | 0.0093 | −0.84 | 0.0042 | −0.66 | 0.11 |
| *TOLLIP* | 9.57 ± 0.16 | 9.59 ± 0.13 | 0.42 | 9.59 ± 0.13 | 9.59 ± 0.13 | 0.91 | −7.52 | 0.57 | 2.34 | 0.27 | 3.57 | 0.20 |
| *MUC5AC/5B* ratio | 1.26 ± 0.88 | 2.57 ± 1.50 | <0.0001 | 2.61 ± 1.52 | 2.54 ± 1.49 | 0.91 | −3.69 | 0.0009 | 6.74 | 0.0024 | 7.08 | 0.016 |
| SARP Cross-Sectional Cohort Microarray mRNA (n = 155)* | | | | | | | | | | | | |
| Gene | Asthma Susceptibility | | | Asthma Severity | | | Baseline Pre-BD $FEV_1$ % Predicted | | Emergency Room or Hospitalization (last 12 months) | | | |
| | Non-Asthma Participants (n = 27) | Patients with Asthma (n = 128) | *P* value† | Non-Severe Asthma (n = 78) | Severe Asthma (n = 50) | *P* value† | β‡ | *P* value‡ (n = 128) | No (n = 77) | Yes (n = 47) | *P* value† | |
| *AP2A2* | 10.4 ± 0.25 | 10.5 ± 0.26 | 0.12 | 10.4 ± 0.26 | 10.5 ± 0.26 | 0.045 | −10.10 | 0.15 | 10.4 ± 0.26 | 10.5 ± 0.26 | 0.11 | |
| *MUC6* | 8.74 ± 0.45 | 8.87 ± 0.48 | 0.16 | 8.84 ± 0.52 | 8.91 ± 0.42 | 0.7 | −6.61 | 0.1 | 8.95 ± 0.49 | 8.78 ± 0.40 | 0.041 | |
| *MUC2* | 8.80 ± 0.87 | 9.04 ± 1.07 | 0.69 | 8.93 ± 1.00 | 9.21 ± 1.16 | 0.11 | −4.45 | 0.01 | 8.85 ± 0.89 | 9.39 ± 1.28 | 0.016 | |
| *MUC5AC* | 14.7 ± 1.39 | 15.1 ± 1.04 | 0.14 | 15.0 ± 1.11 | 15.4 ± 0.88 | 0.087 | −4.05 | 0.03 | 14.9 ± 1.04 | 15.5 ± 0.90 | 0.0094 | |
| *MUC5B* | 11.7 ± 0.86 | 10.6 ± 1.50 | 0.0003 | 10.6 ± 1.44 | 10.5 ± 1.60 | 0.23 | 1.61 | 0.21 | 10.8 ± 1.38 | 10.3 ± 1.62 | 0.076 | |
| *TOLLIP* | 8.22 ± 0.51 | 8.20 ± 0.35 | 0.34 | 8.14 ± 0.26 | 8.30 ± 0.44 | 0.048 | −11.50 | 0.03 | 8.17 ± 0.35 | 8.25 ± 0.35 | 0.44 | |
| *MUC5AC/5B* ratio | 2.98 ± 1.86 | 4.56 ± 2.24 | 0.002 | 4.37 ± 2.25 | 4.84 ± 2.21 | 0.11 | −1.6 | 0.06 | 4.16 ± 2.09 | 5.19 ± 2.26 | 0.017 | |

Definition of abbreviations: pre-BD=pre-bronchodilator; RNA-Seq=RNA sequencing; SARP=Severe Asthma Research Program.

*mRNA expression levels were natural logarithm (ln) and logarithm base 2 (log2) transformed in the SARP longitudinal cohort and cross-sectional cohort, respectively. RNA-Seq of *MUC2* failed quality control and thus not reported in the longitudinal cohort.

†*P* value was generated using a logistic regression model to test correlation between mRNA expression levels and asthma-related phenotypes with adjustment for age, sex, race, body mass index, and batch effect.

‡Regression slope (β) and *P* value were generated using a linear regression model to test correlation between mRNA expression levels and asthma-related phenotypes with adjustment for age, sex, race, body mass index, and batch effect.

**Table 2.**

Correlation of mRNA expression levels of six candidate genes in chr11p15.5 region and T2 cellular biomarkers in bronchial epithelial cells of patients with asthma in SARP.

| **SARP Longitudinal Cohort RNA-Seq (n = 114; Patients with Asthma)*** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Gene** | **Blood Eosinophil Counts** | | **Sputum % Eosinophil** | | **FeNO** | | **Total Serum IgE** | |
| | **β** | ***P* value (n = 114)†** | **β** | ***P* value (n = 84)†** | **β** | ***P* value (n = 113)†** | **B** | ***P* value (n = 111)†** |
| *AP2A2* | 1.26 | 0.0007 | 1.91 | 0.021 | 0.61 | 0.0048 | 0.12 | 0.76 |
| *MUC6* | 0.07 | 0.19 | −0.085 | 0.51 | −0.046 | 0.14 | 0.045 | 0.41 |
| *MUC5AC* | 0.24 | 0.0002 | 0.43 | 0.0014 | 0.13 | 0.0003 | 0.10 | 0.16 |
| *MUC5B* | −0.044 | 0.49 | −0.28 | 0.033 | −0.18 | <0.0001 | −0.064 | 0.32 |
| *TOLLIP* | 0.41 | 0.36 | −0.81 | 0.41 | 0.35 | 0.19 | −0.29 | 0.54 |
| *MUC5AC/5B* ratio | −0.098 | 0.011 | 0.25 | 0.0016 | 0.11 | <0.0001 | 0.056 | 0.15 |
| SARP Cross-Sectional Cohort Microarray mRNA (n = 128; Patients with Asthma) * | | | | | | | | |
| Gene | Blood Eosinophil Counts | | Sputum % Eosinophil | | FeNO | | Total Serum IgE | |
| | β | *P* value (n = 102) † | β | *P* value (n = 62) † | β | *P* value (n = 104) † | β | *P* value (n = 103) † |
| *AP2A2* | −0.09 | 0.73 | 0.21 | 0.68 | 0.2 | 0.11 | −0.19 | 0.48 |
| *MUC6* | −0.22 | 0.13 | 0.088 | 0.75 | 0.03 | 0.67 | 0.35 | 0.01 |
| *MUC2* | −0.11 | 0.083 | 0.092 | 0.45 | 0.11 | 0.0002 | −0.005 | 0.94 |
| *MUC5AC* | 0.10 | 0.14 | 0.26 | 0.033 | 0.13 | <0.0001 | 0.1 | 0.13 |
| *MUC5B* | −0.05 | 0.26 | −0.24 | 0.01 | −0.15 | <0.0001 | −0.087 | 0.056 |
| *TOLLIP* | −0.22 | 0.26 | 0.33 | 0.3 | 0.17 | 0.068 | 0.15 | 0.47 |
| *MUC5AC/5B* ratio | 0.045 | 0.15 | 0.15 | 0.0077 | 0.095 | <0.0001 | 0.06 | 0.047 |

Definition of abbreviations: FeNO=fractional exhaled nitric oxide; RNA-Seq=RNA sequencing; SARP=Severe Asthma Research Program; T2=type-2.

*mRNA expression levels were natural logarithm (ln) and logarithm base 2 (log2) transformed in the SARP longitudinal cohort and cross-sectional cohort, respectively. RNA-Seq of *MUC2* failed quality control and thus not reported in the longitudinal cohort.

†Regression slope (β) and *P* value were generated using a linear regression model to test correlation between mRNA expression levels and T2 cellular biomarkers (logarithm base 10 (log10) transformed) with adjustment for age, sex, race, body mass index, and batch effect.

**Table 3.**

Comparison of results of eQTL and genetic association analyses of 26 candidate SNPs in chr11p15.5 region in SARP and findings in public databases.

| SNP | Gene | Minor / Major Allele | SNP Type | GWAS ($P<1.0\times^{-5}$) [21] | GWAS of Asthma [12] | eQTL reported | eQTL in BEC in the SARP Longitudinal Cohort† | | | Asthma Severity‡ | LD Block§ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Effect Allele | *MUC5AC* β (*P* value) | *MUC5B* β (*P* value) | *TOLLIP* β (*P* value) | OR (P value) | |
| rs7104956 | *AP2A2* | G/C | intron | C~asthma ($2.4\times10^{-7}$) [5] | NA | NS | G | −0.39 (0.030) | 0.13 (0.48) | 0.03 (0.89) | 0.98 (0.86) | |
| rs7934606 | *MUC2* | A/G | intron | A~IIPs ($6.9\times10^{-34}$) [13] A~IPF ($3.8\times10^{-6}$) [14] | NS | NS | A | −0.10 (0.49) | 0.04 (0.77) | −0.05 (0.71) | 0.96 (0.70) | 1 |
| rs6421972 | *MUC2* | A/G | synon. | A~IIPs ($1.9\times10^{-21}$) [13] | NS | NS | A | −0.12 (0.42) | −0.02 (0.90) | −0.06 (0.67) | 0.95 (0.60) | |
| rs35225972 | *MUC2*-MUC5AC | G/A | intergenic | A~asthma ($4\times10^{-10}$) [11] | NA | NS | G | −0.32 (0.0071) | 0.32 (0.0080) | 0.051 (0.68) | 0.99 (0.93) | 2 |
| rs12804326 | *MUC2*-MUC5AC | T/C | intergenic | NA | C ($4.9\times10^{-6}$) | NS | T | −0.31 (0.017) | 0.34 (0.0092) | 0.14 (0.29) | 0.86 (0.22) | |
| rs10902095 | *MUC2*-MUC5AC | T/C | intergenic | NA | C ($5.2\times10^{-6}$) | NS | T | −0.31 (0.017) | 0.34 (0.0092) | 0.14 (0.29) | 0.87 (0.25) | |
| rs12365253 | *MUC2*-MUC5AC | T/C | intergenic | NA | C ($5.1\times10^{-6}$) | NS | T | −0.30 (0.023) | 0.33 (0.012) | 0.13 (0.32) | 0.86 (0.22) | |
| rs12365278 | *MUC2*-MUC5AC | T/C | intergenic | NA | C ($1.7\times10^{-6}$) | NS | T | −0.28 (0.036) | 0.32 (0.014) | 0.15 (0.25) | 0.77 (0.24) | |
| rs10794296 | *MUC2*-MUC5AC | T/C | intergenic | NA | C ($4.7\times10^{-6}$) | NS | T | −0.31 (0.019) | 0.28 (0.037) | 0.11 (0.43) | 0.86 (0.21) | |
| rs12788104 | *MUC2*-MUC5AC | A/G | intergenic | G~adult onset asthma ($1.4\times10^{-8}$) [8] G~asthma ($2\times10^{-18}$) [9] G~asthma ($2\times10^{-10}$) [10] | NA | NS | A | −0.32 (0.0062) | 0.28 (0.016) | −0.01 (0.94) | 1.00 (0.98) | |
| rs6421966 | *MUC2*-MUC5AC | G/T | intergenic | NA | T ($7.2\times10^{-6}$) | NS | G | −0.31 (0.015) | 0.23 (0.071) | 0.01 (0.93) | 0.84 (0.16) | |
| rs7130988 | *MUC2*-MUC5AC | G/A | intergenic | NA | A ($4.0\times10^{-6}$) | NS | G | −0.31 (0.015) | 0.23 (0.071) | 0.01 (0.93) | 0.84 (0.16) | |

| SNP | Gene | Minor / Major Allele | SNP Type | GWAS ($P<1.0\times^{-5}$) [21] | GWAS of Asthma [12] | eQTL reported | eQTL in BEC in the SARP Longitudinal Cohort† | | | | Asthma Severity‡ | LD Block§ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Effect Allele | *MUC5AC* β (*P* value) | *MUC5B* β (*P* value) | *TOLLIP* β (*P* value) | OR (P value) | |
| rs11603634 | *MUC2*-MUC5AC | A/G | intergenic | G~moderate-to-severe asthma ($2.3\times10^{-8}$) [6] | NA | G~MUC5AC↑ ($2.5\times10^{-5}$) in BEC [6] | A | −0.36 (0.0064) | 0.21 (0.12) | −0.08 (0.56) | 0.98 (0.81) | |
| rs7112954 | *MUC2*-MUC5AC | T/C | intergenic | NA | C ($5.2\times10^{-6}$) | NS | T | −0.29 (0.025) | 0.21 (0.12) | 0.03 (0.81) | 0.85 (0.22) | |
| rs28415845 | *MUC2*-MUC5AC | T/C | intergenic | C~adult onset asthma ($2.9\times10^{-13}$) [7] | NA | NS | T | −0.28 (0.023) | 0.24 (0.055) | 0.02 (0.90) | 0.99 (0.96) | |
| rs28403537 | *MUC5AC* | T/C | missense | T~IPF ($3.2\times10^{-9}$) [14] | NA | NS | T | 0.06 (0.87) | −0.29 (0.44) | −0.22 (0.56) | 0.88 (0.60) | |
| rs35288961 | *MUC5AC* | T/G | intron | T~IPF ($3.7\times10^{-6}$) [14] | NA | NS | T | −0.17 (0.40) | 0.25 (0.21) | −0.40 (0.043) | 1.08 (0.54) | |
| rs34595903 | *MUC5AC*-MUC5B | T/C | intergenic | C~IPF ($2.4\times10^{-6}$) [14] | NA | NS | T | 0.26 (0.071) | −0.43 (0.0029) | 0.11 (0.44) | 1.03 (0.76) | 3 |
| rs2672794 | *MUC5B* | T/C | promoter | C~IPF ($1.9\times10^{-7}$) [14] | NA | NS | T | 0.28 (0.041) | −0.33 (0.018) | 0.004 (0.98) | 0.97 (0.75) | |
| rs35705950 | *MUC5B* | T/G | promoter | T~IIPs ($7.2\times10^{-95}$) [13] T~IPF ($4.6\times10^{-31}$) [14] T~IPF ($1.1\times10^{-66}$) [15] T~IPF ($2.4\times10^{-50}$) [16] T~IPF ($1\times10^{-203}$) [17] T~IPF ($5\times10^{-29}$) [18] T~IPF ($2\times10^{-65}$) [19] | NA | T~MUC5B↑ ($1.1\times10^{-13}$) in lung [22] | T | −0.41 (0.15) | 0.15 (0.60) | −0.45 (0.11) | 1.02 (0.89) | |
| rs35619543 | *MUC5B* | T/G | promoter | T~IPF ($1.5\times10^{-8}$) [14] | NA | NS | T | −0.33 (0.035) | 0.23 (0.15) | −0.14 (0.37) | 1.09 (0.47) | |
| rs868903 | *MUC5B* | T/C | promoter | C~IIPs ($5.7\times10^{-10}$) [13] | NS | NS | T | −0.17 (0.21) | −0.10 (0.47) | −0.14 (0.27) | 0.90 (0.30) | |
| rs3829223 | *TOLLIP* | T/C | intron | T~IIPs ($7.2\times10^{-6}$) [13] | NS | NS | T | 0.15 (0.28) | −0.32 (0.021) | −0.23 (0.096) | 1.01 (0.93) | 4 |

| SNP | Gene | Minor / Major Allele | SNP Type | GWAS ($P<1.0\times^{-5}$) [21] | GWAS of Asthma [12] | eQTL reported | eQTL in BEC in the SARP Longitudinal Cohort† | | | | Asthma Severity‡ | LD Block§ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Effect Allele | *MUC5AC* β (*P* value) | *MUC5B* β (*P* value) | *TOLLIP* β (*P* value) | OR (P value) | |
| rs111521887 | *TOLLIP* | G/C | intron | G~IPF ($2.2\times10^{-12}$) [16] | NA | G~TOLLIP↓ ($3.0\times10^{-4}$) in lung [16] | G | 0.11 (0.57) | −0.20 (0.30) | 0.10 (0.60) | 1.24 (0.094) | |
| rs5743894 | *TOLLIP* | C/T | intron | C~IPF ($1.4\times10^{-12}$) [16] | NA | C~TOLLIP↓ ($2.9\times10^{-5}$) in lung [16] | C | 0.11 (0.57) | −0.20 (0.30) | 0.10 (0.60) | 1.23 (0.10) | |
| rs5743890 | *TOLLIP* | C/T | intron | T~IPF ($3.4\times10^{-11}$) [16] | NS | C~TOLLIP↓ (0.097) in lung [16] | C | 0.21 (0.27) | −0.17 (0.36) | −0.54 (0.0041) | 0.86 (0.25) | |

Definition of abbreviations: BEC=bronchial epithelial cells; eQTL=expression quantitative trait locus; GWAS=genome-wide association studies; IIPs: idiopathic interstitial pneumonias; IPF: idiopathic pulmonary fibrosis; LD: linkage disequilibrium; NS: non-significant; NA: non-available; SARP=Severe Asthma Research Program.

†Only entries with eQTL *P*<0.05 in BEC in the SARP longitudinal cohort were included. Regression slope (β) and *P* value were generated using a linear regression model, adjusted for age, sex, race, BMI, and batch effect.

‡Asthma Severity: odds ratio (OR) and *P* value were generated using an additive logistic regression model to test genetic association of asthma severity (426 severe asthma vs. 531 non-severe asthma) in SARP non-Hispanic White adults (age≥12 years old), adjusted for age, sex, and the first five components from the multidimensional scaling analysis of genome.

§LD Block: LD was estimated with 95% confidence intervals of D' to define LD blocks of 26 SNPs based on 1,016 non-Hispanic Whites in SARP using Haploview [32].