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Genetic analyses identify *GSDMB* associated with asthma severity, exacerbations, and antiviral pathways

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Abstract

Background: Chr17q12-21.2 region is the strongest and most consistently associated region with asthma susceptibility. The functional genes or single nucleotide polymorphisms (SNPs) are not obvious due to linkage disequilibrium.

Objectives: Whole-genome sequence and RNAseq from human bronchial epithelial cells (BEC) were comprehensively investigated to dissect functional genes/SNPs for asthma severity in the Severe Asthma Research Program (SARP).

Methods: eQTL analysis (n=114), correlation analysis (n=156) of gene expression and asthma phenotypes, and pathway analysis were performed in BEC and replicated. Genetic association for asthma severity (426 severe vs. 531 non-severe asthma) and longitudinal asthma exacerbations (n=273) was performed.

Results: Multiple SNPs in *GSDMB* associated with asthma severity (odds ratio>1.25) and longitudinal asthma exacerbations ($p<0.05$). eQTL analyses identified multiple SNPs associated with expression levels of *PGAP3, GSDMB*, or *GSDMA* ($3.1x10^{-9}<p<1.8x10^{-4}$). Higher expression levels of *GSDMB* correlated with asthma and greater number of exacerbations ($p<0.05$). Expression levels of *GSDMB* correlated with genes involved in interferon signaling, MHC class I antigen presentation, and immune system pathways (FDR-$p<0.05$). rs1031458 and rs3902920 in *GSDMB* colocalized with interferon regulatory factor (IRF) binding sites and associated with *GSDMB* expression, asthma severity, and asthma exacerbations ($p<0.05$).

Conclusions: By using a unique set of gene expression data from lung cells obtained using bronchoscopy from comprehensively characterized asthma subjects, we show that SNPs in *GSDMB* associated with asthma severity, exacerbations, and *GSDMB* expression levels. Furthermore, its expression levels correlated with asthma exacerbations and antiviral pathways. Thus, *GSDMB* is a functional gene for both asthma susceptibility and severity.

Capsule summary

By using a unique dataset of gene expression from lung cells of asthmatics, we show strong evidence for *GSDMB* as a gene for asthma severity and asthma exacerbations probably through antiviral pathways.

Keywords

Antiviral pathways; asthma exacerbations; asthma severity; eQTL; genetics; *GSDMA*; *GSDMB*; *PGAP3*; whole-genome sequence; RNAseq

INTRODUCTION

Asthma is a common inflammatory airway disease. *ORMDL3* in chr17q12-21.2 region was the first gene identified through genome-wide association study (GWAS) of asthma.[1] Since then, GWAS, candidate gene replication, and gene expression studies have consistently identified or confirmed SNPs in multiple genes in this region that are associated with asthma susceptibility, including *PGAP3*,[2–4] *ERBB2*,[5] *IKZF3*,[6–9] *ZPBP2*,[10–11] *GSDMB*,[1,5,8,12–20] *ORMDL3*,[11,21–25] and *GSDMA*.[9,14–15,26] SNPs in *IKZF3*[27] *ZPBP2*,[28] *GSDMB*,[10,29–30] and

PSMD3[29] have also been associated with allergic responses. However, partially due to linkage disequilibrium (LD), it has been difficult to determine the specific genes or SNPs responsible for those association. In addition, most published GWAS of asthma has tested the association of SNPs with asthma susceptibility (mild or severe asthma vs. healthy controls), not asthma severity. To analyze asthma severity, we performed a genetic association analysis for severe asthma compared to non-severe asthma and asthma exacerbations longitudinally over a 3 year period.

Autoimmune diseases (AD) arise from abnormal immune responses to self-antigens. SNPs in *ERBB2*,[31] *IKZF3*,[32–44] *ZPBP2*,[45–46] *GSDMB*,[47–58] and *GSDMA*[59–60] have been associated with a variety of AD. In a previously published GWAS, we were the first to report that the opposite risk alleles in *IL13, TNIPI, HLA-DRA*, and *GSDMB* associated with asthma and AD.[61] In this study, we comprehensively compared all the GWAS-identified SNPs associated with asthma, allergy, and AD in chr17q12-21 region to reveal genetic effects on the immunopathogenesis of asthma, allergy, and AD.

In a recent review, genetic association, expression quantitative trait loci (eQTL), and epigenetics of 17 SNPs in chr17q12-21.2 region with asthma have been summarized.[62] Proximal (*PGAP3-ERBB2*), core (*IKZF3-ZPBP2-GSDMB-ORMDL3*), and distal (*GSDMA*) regions have been suggested as independent regions associated with asthma.[62]

In order to delineate the functional genes/SNPs for asthma severity in this region, we utilized a unique dataset of lung gene expression data obtained from bronchial brushing during investigational bronchoscopy in extensively characterized patients with current asthma plus healthy controls. We hypothesize that combing SNP with RNA gene expression data from lung cells of asthmatics, we will be able to determine the functional asthma genes/SNPs in this complicated chromosomal region.

METHODS

Study subjects

SARP is a currently active multicenter program funded for the last 18 years by the NHLBI. Mild to severe subjects with asthma (enriched for severe) and a subset of controls have been extensively studied using standardized protocols. The earlier SARP cohort was cross-sectional (n=1,644). In a subset of subjects with mild to severe asthma, RNA was isolated from epithelial cells (BEC; n=155) that were obtained from brush biopsies (Table I and Table E1).[63–65] The current SARP cohort is an ongoing longitudinal study (n=714).[66–68] Bronchoscopy was performed on a subset of the longitudinal cohort to obtain epithelial cells from brush biopsies (n=156) for RNAseq (Table I and Table E1). All studies were approved by the appropriate institutional review board at the participating sites including informed consent.

Statistical analysis

Selection of SNPs and RNAseq Data.—Whole genome sequencing (WGS) in SARP (n=1,888; version Freeze 6; dbGaP accession: phs001446) was performed through NHLBI-sponsored TOPMed Program (www.nhlbiwgs.org). Standard quality control (QC) was

performed. All SNPs in chr17q12-21.2 region were extracted (hg38: *PPP1R1B* to *CSF3*; chr17:39,626,924-40,017,813) in the longitudinal cohort with WGS using PLINK 1.9 software,[69] and further QC were performed as described.[61,70] Similarly, SNPs were extracted from the cross-sectional cohort with GWAS data and imputed based on TOPMed reference panel using the Michigan Imputation Server.[71]

RNAseq data from BEC in the longitudinal cohort were extracted for 14 candidate genes (except for *ZPBP2* and *LRRC3C* which failed QC) in chr17q12-21.2 region. In brief, Illumina HiSeq RNAseq reads were quality filtered and mapped to human genome hg38 using STAR package.[72] Read counts were regularized logarithm transformed using DESeq2 package.[73] The RNAseq data will be deposited and accessible through GEO (www.ncbi.nlm.nih.gov/geo/). Agilent Whole Human Genome Microarray expression data of these 16 genes were extracted from BEC in the cross-sectional cohort as described.[74–75] The microarray expression data have been deposited and can be accessed through GSE63142 and GSE43696.[74,76–77]

Genetic Association Analysis.—Logistic or linear regression, assuming a genetic additive model, was used for genetic association analysis of asthma severity (426 severe asthma vs. 531 non-severe asthma) and the number of exacerbations (n=273) due to asthma in three years in non-Hispanic White adults (age>12 years old) in the longitudinal cohort (Table I), adjusted for age, sex, and the first five components from the multidimensional scaling analysis of genome.

We first investigated a set of 48 candidate SNPs identified by previous GWAS of asthma, allergy, and AD (NHGRI-EBI GWAS catalog;[78] www.ebi.ac.uk/gwas/) incorporated in UCSC genome browser (genome.ucsc.edu; accessed on August 12, 2019)[79] for association with asthma severity and longitudinal exacerbations in SARP (Figure 1). To reduce multiple tests due to SNPs with strong LD, the numbers of independent tests were calculated using GEC.[80] 14.4 independent tests of 48 candidate SNPs were indicated by GEC, and thus SNPs with p-value<0.0035 (0.05/14.4 tests) were considered significant. SNPs with p-value<0.05 were considered as nominally significant. From all sequenced SNPs in the chr17q12-21.2 region, we extracted 1,266 common SNPs (MAF≥0.01) to test for association and p-value<0.05 was considered as nominally significant due to relatively small sample size. Note that all of the 48 candidate SNPs were included in the set of 1,266 common SNPs. LD was estimated with 95% confidence intervals of D' to define LD blocks and LD plots of candidate SNPs in chr17q12-21.2 region were generated separately for 1,016 non-Hispanic Whites and 622 African Americans (Table I) using Haploview.[81]

eQTL Analysis.—A linear additive genetic model was used to test the association between SNPs and inverse normalized expression data as described before.[74–75] The longitudinal and cross-sectional cohorts were used as discovery and replication datasets, respectively (Figure 1). Significant eQTL SNPs identified in the lung tissue (n=383) from Genotype-Tissue Expression (GTEx) database[26] were also evidence for replication (Figure 1). In the longitudinal cohort with WGS and RNAseq in BEC (n=114), 252.6 independent tests of 862 common SNPs (MAF≥0.05) in chr17q12-21.2 region were indicated by GEC,[80] and thus, SNPs with p-value<1.98×10^{-4} (0.05/252.6 tests) were considered as significant eQTL SNPs.

SNPs with p-value<0.05 were considered as nominally significant. Conditional eQTL analysis of *PGAP3, GSDMB*, and *GSDMA* in the longitudinal cohort was performed to identify independent eQTL SNPs by stepwise adjusting the most significant eQTL SNP.

Colocalization Analysis.—To test whether the same SNP (n=862) is responsible for the genetic association of asthma severity and eQTL of *PGAP3, GSDMB*, or *GSDMA* in the longitudinal cohort (Figure 1), a Bayesian-based colocalization analysis was performed using coloc package.[82] A posterior probability of 75% or greater was considered as strong evidence of colocalization. Colocalization analysis of SNPs associated with asthma severity or longitudinal asthma exacerbations and with gene expression of *PGAP3, GSDMB*, or *GSDMA* in the longitudinal cohort (Figure 1) was also performed through conditional eQTL analysis by adjusting the most significant SNP associated with asthma severity or longitudinal asthma exacerbations.

Correlation Analysis of Gene Expression and Asthma Phenotypes.—Correlation analysis of gene expression and asthma-related phenotypes was performed as described (Figure 1).[74–75] In brief, a generalized linear model was used to test the correlation between expression levels of 16 candidate genes and asthma-related phenotypes with adjustment of age, sex, race (dummy variables for non-Hispanic Whites and African Americans), BMI, and batch effect. P-value<0.05 was considered as nominally significant.

Pathway Analysis.—Correlation analysis of gene expression levels of 16,068 genes in the longitudinal cohort or 19,567 genes in the cross-sectional cohort was performed using Spearman's rank correlation. The genes with expression levels significantly correlated with *PGAP3, GSDMB*, or *GSDMA* ($p<0.05/16{,}067=3.1\times10^{-6}$ in the longitudinal cohort and $p<0.05/19{,}566=2.5\times10^{-6}$ in the cross-sectional cohort) were input into Reactome software for pathway analysis[83] (Figure 1). Enriched biological pathways were identified using a hypergeometric distribution test with false discovery rate (FDR) adjusted p-value<0.05.

IRF Binding Site Analysis.—Interferon regulatory factor (IRF) binding sites were checked for *GSDMB* based on ENCODE database (Figure 1).[84] Genetic association and eQTL analyses were performed for two common SNPs and four rare SNPs (MAF<0.01) in the identified IRF binding sites of *GSDMB*.

RESULTS

Genetic Association Analysis

16 candidate genes in chr17q12-21.2 region (Figure E1) were selected based on the published GWAS of asthma, allergy, or AD.[78] To elucidate shared genetic variants for immune diseases, 48 SNPs in this region identified through GWAS of asthma, allergy, and AD[78–79] or associated with asthma as reported by Stein *et al.*[62] were investigated (Table II).

Most of the SNPs previously associated with asthma susceptibility were associated with asthma severity at the nominal p-value of 0.05 (Table II). rs2305479 and rs62067034 in *GSDMB* were significantly associated with asthma severity after multiple-test adjustment (odds ratio=1.34; p=0.0029<0.0035). When testing 1,266 common SNPs, several

independent signals were associated with asthma severity though no SNP reached a more stringent significance ($p<0.05/1266$) (Table E2), including five SNPs in *GSDMB* (odds ratio>1.3; $p<0.0035$) with the risk alleles associated with increased *GSDMB* expression.

Most of the SNPs previously associated with asthma susceptibility were also associated with longitudinal asthma exacerbations at the nominal p-value of 0.05 (Table II). rs2517955 in *PGAP3* was significantly associated with longitudinal asthma exacerbations after multiple-test adjustment ($p=0.0034$). When testing 1,266 common SNPs, several independent signals were associated with longitudinal asthma exacerbations though no SNP reached stringent significance ($p<0.05/1266$) (Table E3), including four SNPs in *PGAP3-ERBB2* region ($p<0.0035$) with the risk alleles associated with increased *PGAP3* expression.

Multiple SNPs in this region were associated with asthma, allergy, and AD, however, the risk alleles were opposite between asthma and AD (Table II). For example, the G allele of rs907092 in *IKZF3* was the risk allele for asthma ($p<5x10^{-8}$)[7–8] and asthma severity ($p=0.027$), and associated with higher expression levels of *GSDMB* ($p=3.7x10^{-4}$) and *PGAP3* ($p=7.9x10^{-4}$), but was the protective allele for primary biliary cholangitis (PBCh) ($p<5x10^{-8}$).[38] The G allele of rs2305480 (a missense mutation in *GSDMB*) was the risk allele for asthma ($p<5x10^{-8}$),[14–15] asthma severity ($p=0.015$), longitudinal asthma exacerbations ($p=0.0086$) and associated with higher expression levels of *GSDMB* ($p=2.5x10^{-5}$), but was the protective allele for rheumatoid arthritis (RA) and ulcerative colitis (UC) ($p<5x10^{-8}$).[48,57] The A allele of rs3894194 (a missense mutation in *GSDMA*) was the risk allele for asthma ($p<5x10^{-8}$)[14–15] and associated with lower expression levels of *GSDMA* ($p=4.3x10^{-4}$), but was the protective allele for systemic sclerosis (SS) ($p<5x10^{-8}$).[59] All 48 candidate SNPs identified by previous GWAS (Table II) were common SNPs (MAF>0.01), and thus, belonged to 1,266 common SNPs analyzed in this study. When ranking genetic association of asthma severity p-values of 1,266 SNPs, 35 (73%), 6 (13%), 3 (6%), and 4 (8%) of 48 candidate SNPs were distributed in the 1st to 4th quartile, respectively.

eQTL Analysis and Colocalization Analysis

Expression of 14 genes (except *ZPBP2* and *LRRC3C*) in the longitudinal cohort (n=114 BEC) and 16 gene in the cross-sectional cohort (n=120 BEC) passed QC (Table I and Table E1). LD pruning ($r^2\geq0.8$) of 862 common SNPs (MAF≥0.05) belonging to these 16 candidate genes generated 273 SNPs. The complete eQTL results of 862 SNPs were summarized in Table E4. 26 of 273 SNPs were significantly associated with the gene expression levels of *PGAP3, GSDMB*, or *GSDMA*, but not associated with the other genes in the longitudinal cohort (Table III and Table E4–E5). The eQTL findings of 26 SNPs in the longitudinal cohort were generally replicated in BEC in the cross-sectional cohort at nominal p-value of 0.05 (Table E6). Considering stringent replication ($p<0.05/26=1.9x10^{-3}$), 16 of 26 SNPs in *PGAP3* or *GSDMB* were replicated in BEC in the cross-sectional cohort; 21 of 26 SNPs in *PGAP3, GSDMB*, or *GSDMA* were replicated in GTEx lung tissue (Table III); all together, 22 of 26 SNPs were replicated. Three and six LD blocks were formed for these 26 SNPs in non-Hispanic Whites and African Americans, respectively (Table III, Figure E2–E3). SNPs in *PPP1R1B, PGAP3*, and *ERBB2* were associated with *PGAP3*

expression. SNPs in *IKZF3* region were associated with the expression levels of *PGAP3, GSDMB*, or *GSDMA*. SNPs in *ZPBP2, GSDMB*, and *ORMDL3* were associated with *GSDMB* expression. SNPs in *GSDMA* were associated with *GSDMA* expression. Most of these 26 eQTL SNPs were associated with asthma severity or longitudinal asthma exacerbations at a nominal p-value of 0.05 (Table E7).

Five and six LD blocks were identified for 48 GWAS-identified SNPs in non-Hispanic Whites and African Americans, respectively (Table II, Figure E4–E5). Significant eQTL SNPs ($p<0.0035$) were associated with the expression levels of three genes (*PGAP3, GSDMB*, or *GSDMA*) in the longitudinal cohort and were generally replicated in the cross-sectional cohort at nominal p-value of 0.05 (Table II and Table E8). GTEx lung tissue eQTL in this region identified four genes (*PGAP3, GSDMB, ORMDL3*, and *GSDMA*) (Table II and III).

Conditional eQTL analysis was performed by stepwise adjusting the most significant eQTL SNP (Table E9), and indicated that two SNPs (rs2517954 in *PGAP3* and rs114211283 in *IKZF3*), two SNPs (rs11657449 in *ZPBP2-GSDMB* and rs3794712 in *PPP1R1B*), and one SNP (rs3859193 in *GSDMA*) were independent eQTL SNPs for *PGAP3, GSDMB*, and *GSDMA*, respectively.

Colocalization analysis[82] of the signals from genetic association of asthma severity and eQTL was performed, and indicated no significant colocalization SNP based on the criterion of posterior probability>75% (Table E10). rs2517954 in *PGAP3*, rs11657449 in *ZPBP2-GSDMB*, and rs2941522 in *GRB7-IKZF3* were top colocalization SNPs for *PGAP3, GSDMB*, and *GSDMA*, respectively (Table E11). Colocalization analysis between SNPs associated with asthma severity or longitudinal asthma exacerbations and gene expression of *PGAP3, GSDMB*, and *GSDMA* was also performed through conditional eQTL analysis by adjusting the most significant SNP associated with asthma severity or longitudinal asthma exacerbations (Table E12, Table II). With adjustment of rs2952156 in *ERBB2*, rs2305479 in *GSDMB*, and rs3902025 in *GSDMA*, all eQTL SNPs for *PGAP3* (except for rs114211283 in *IKZF3*), for *GSDMB* (except for two SNPs in *PPP1R1B* and *ZPBP2-GSDMB*), and for *GSDMA* became non-significant. For example, the association between *GSDMB* expression and rs11657449 in *ZPBP2-GSDMB* or rs3794712 in *PPP1R1B* was weakened when adjusting for rs2305479, indicating that rs2305479 partly accounted for the eQTL association but not completely. In summary, the colocalization analyses did not show strong evidence for colocalization.

Expression Analysis and Pathway Analysis

The risk alleles associated with asthma, asthma severity, and longitudinal asthma exacerbations were associated with higher expression levels of *PGAP3* and *GSDMB* or the lower expression levels of *GSDMA* (Table II), which indicated that expression levels of *PGAP3, GSDMB*, and *GSDMA* may be correlated with asthma phenotypes.

Correlation analysis of gene expression (*PGAP3, GSDMB*, and *GSDMA*) and asthma phenotypes was performed in BEC in the longitudinal cohort (n=156) and replicated in BEC (n=155) in the cross-sectional cohort (Table IV). Higher expression levels of *GSDMB* were

correlated with asthma (p=0.05), greater number of exacerbations in the last 12 months (p=0.02), and higher reduction of ACQ-6 after steroid treatment (p=0.0008) in the longitudinal cohort. Higher expression levels of *GSDMB* were correlated with emergency room (ER) visits or hospitalizations due to asthma in the last 12 months (p=0.03) in the cross-sectional cohort. Other asthma-related phenotypes were not correlated with expression levels of *PGAP3, GSDMB*, or *GSDMA* (Table E13), except that higher expression levels of *GSDMB* were correlated with higher FeNO (p=0.03) in the longitudinal cohort. Although correlation analysis was focused on *PGAP3, GSDMB*, and *GSDMA*, the other 13 genes were also analyzed (Table E14–E15). Higher expression of *PNMT* and lower expression of *CSF3* were associated with asthma susceptibility in BEC in the longitudinal and cross-sectional cohorts.

Pathway analyses were performed on the genes with expression levels significantly correlated with *PGAP3, GSDMB*, or *GSDMA*. No biological pathways were identified for the genes correlated with *PGAP3* or *GSDMA* after FDR adjustment (data not shown). 435 and 677 genes were positively and negatively correlated with *GSDMB* ($p<3.1x10^{-6}$) in BEC in the longitudinal cohort, among which 636 genes were replicated in BEC in the cross-sectional cohort ($p<0.05$) (Table E16). Pathway analysis[83] was performed on 1,112 and 462 genes with expression levels significantly correlated with *GSDMB* expression in BEC in the longitudinal cohort ($p<3.1x10^{-6}$) and cross-sectional cohort ($p<2.5x10^{-6}$), respectively. Expression levels of *GSDMB* were correlated with genes involved in interferon alpha/beta/gamma signaling, MHC class I antigen presentation, and immune system pathways (FDR-$p<0.05$) (Table V and Table E17).

IRF Binding Site Analysis

Interferon regulatory factor (IRF) binding sites were checked for *GSDMB* and two regions were identified based on ENCODE database (Figure E6).[84] One IRF1/2 biding site was located at 5'UTR-exon 1-intron 1 region of *GSDMB* (Figure E7) and one IRF4 biding site was located at intron 2 of *GSDMB* (Figure E8). Two common SNPs and four rare SNPs were found in these two IRF biding sites based on SARP WGS (Table VI). Two common SNPs (rs1031458 and rs3902920) were associated with *GSDMB* expression, asthma severity, and longitudinal asthma exacerbations ($p<0.05$), making them potential functional SNPs.

T allele of rs1031458 or C allele of rs3902920 were risk alleles for asthma severity and longitudinal asthma exacerbations (Table VI), and they were also associated with early onset of asthma ($p<0.005$) (Table E18) especially atopic early onset (age onset of asthma<6 yrs) asthma ($p<0.00001$) (Table E19 and Figure 2). Similarly, most of the top 10 SNPs associated with asthma severity (including rs3902920; Table E2) were also associated with asthma severity in the subjects with early onset asthma (onset<6 yrs) (Table E20). rs1031458 and rs3902920 were in strong LD ($r^2\geq0.8$) with multiple neighboring SNPs (Table II–III) in non-Hispanic Whites (Table E21). In African Americans, rs1031458 and rs3902920 were in strong LD with three (rs921650, rs7216389, and rs201413617) and zero neighboring SNPs, respectively.

In summary, by using a unique set of gene expression data from lung cells of asthmatics obtained using investigative bronchoscopy and by performing comprehensive genetic association, expression correlation, eQTL, and pathway analyses, we have narrowed down chr17q12-21.2 region (16 candidate genes; 390 kbp) to two SNPs in *GSDMB* associated with asthma severity and asthma exacerbations potentially through antiviral pathways (Figure 1).

DISCUSSION

Almost all the SNPs identified by previous GWAS in *GSDMB* now show to be associated with asthma severity and longitudinal asthma exacerbations, indicating that SNPs in *GSDMB* are associated with asthma susceptibility, asthma severity, and asthma exacerbations. Asthma and AD share extensive immunological pathways, however, the risk alleles of the same associated SNPs in this region are consistently opposite for asthma and AD, which may indicate distinct immunopathogenesis processes. In addition to SNPs with MAF≥0.01, we also investigated rare SNPs (MAF<0.01; n=4,006) for association with asthma severity. 14 rare SNPs were associated with asthma severity at nominal p-value of 0.05 with large effect size (2.9<odds ratio<12) (Table E22). Replication of these rare SNPs is needed in larger cohorts with sequence data and asthma phenotypes. In conclusion, findings from genetic association of asthma susceptibility, asthma severity, and asthma exacerbations in this region are generally consistent, however, genetic association analysis can not narrow down the 16 candidate genes due to strong and complicated LD structure in this region.

Gene expression is dependent on cell type or tissue, time, and environmental factors such as disease status. It is critical that cells are obtained from the appropriate organ (lung for asthma) and from living subjects with the disease being investigated instead of from surgical specimens (usually from cancer patients) or autopsy specimens. Even findings of eQTL analyses in lung cells are not always consistent (Table E23). The most significant eQTL genes were *GSDMA* followed by *GSDMB* and *ORMDL3* in two eQTL studies in lung tissue.[26,85] Nicodemus-Johnson *et al*. identified *ORMDL3* but not *GSDMB* in an eQTL analysis in BEC.[4] Our eQTL analysis in BEC in both longitudinal and cross-sectional cohorts[74] identified *GSDMB* but not *ORMDL3*.

Similarly, a recent genetic association and eQTL study has shown that eQTL SNPs for *GSDMB* (but not *ORMDL3*) in BEC play a major role in childhood asthma in African Americans.[86] BEC obtained from brush biopsies are mainly composed of epithelial cells, although small proportion of basal cells and immune cells also exist. A flow cytometry study showed that 95% to 97% of the cells from bronchial brushings were epithelial cells.[87] In this study, cell populations were not available for every subject, and thus, were not adjusted. Future eQTL and expression analyses by adjusting cell composition or single-cell RNAseq may reveal interesting results.

SNPs in *PGAP3-ERBB2* region were associated with *PGAP3* expression and longitudinal asthma exacerbations. In a previous GWAS, rs2941504 in *PGAP3* has been associated with asthma.[2] Another GWAS has identified rs2952156 in *ERBB2* associated with asthma[5] and

PGAP3 expression in lung tissue.[26] Thus, SNPs in *PGAP3-ERBB2* are associated with asthma phenotypes by up-regulating *PGAP3* gene expression. The first GWAS of asthma has identified rs7216389 in *GSDMB* associated with childhood asthma and the expression levels of *ORMDL3* and *GSDMB* in lymphoblastoid cell lines.[1] In this study, rs7216389 was significantly associated with *GSDMB* expression ($p=1.7x10^{-4}$) but not *ORMDL3* (p=0.22) in BEC. Thus, SNPs in *ZPBP2-GSDMB-ORMDL3* are associated with asthma phenotypes by up-regulating *GSDMB* gene expression. rs3894194 in *GSDMA* has been associated with asthma[14–15] and the expression levels of *GSDMA* in lung tissue.[26] In this study, rs3894194 was significantly associated with *GSDMA* expression ($p=4.3x10^{-4}$). Thus, SNPs in *GSDMA* are associated with asthma phenotypes by down-regulating *GSDMA* gene expression. Interestingly, SNPs in *IKZF3* were not consistently associated with a specific gene expression, instead, associated with the expression levels of *PGAP3, GSDMB*, or *GSDMA*, which may indicate long-distance gene expression regulation. Interaction between gene regulatory elements and genes shown by GeneHancer[88] also indicated *IKZF3* was involved in complicated long-distance regulation of *GSDMB, GSDMA, ORMDL3*, and *ERBB2* (Figure E9). In summary, our findings confirm the hypothesis that there are proximal, core, and distal regions independently associated with asthma.[62] In addition, *IKZF3* forms a long-distance regulation region. More importantly, we narrowed down 16 candidate genes to three genes (*PGAP3, GSDMB*, and *GSDMA*).

We attempted to identify functional SNPs using colocalization and conditional eQTL analyses. rs2517954 for *PGAP3* and rs11657449 for *GSDMB* were identified by both colocalization analysis and conditional eQTL analysis, though the posterior probability of colocalization was not high. The probable reason is that the signals of genetic association are not strong due to sample size, and thus, eQTL signals drive the colocalization findings in SARP. Colocalization analysis through conditional eQTL analysis (Table E12) further indicates that the colocalization analysis based on the Bayesian approach does not show strong evidence for colocalization.

Previous studies have shown inconsistent relationship between gene expression in this region and asthma susceptibility.[62] The mRNA levels of *ORMDL3* in lymphoblastoid cell lines have not been significantly different in children with or without asthma.[1] An immunohistochemistry study has found that GSDMB protein levels are significantly higher in subjects with asthma than controls.[89] In this study, higher mRNA levels of *GSDMB* were correlated with asthma and asthma exacerbations, though the correlation was not strong and not always consistently significant. Although our findings are based on relevant tissues (BEC) in relevant subjects (healthy controls, non-severe and severe asthma), subjects involved in this study are all adults (age≥12 years old). Typical of adult asthma cohorts, the SARP cohort consists of those with early onset of asthma and those with older age onset.[63,67] Since asthma is often an early-onset disease, expression or eQTL analyses in children would be interesting but, of course, research bronchoscopies are not performed in children. In this study, gene expression correlation and eQTL analyses were performed in all SARP subjects with mixed races to increase sample size and power. Although gene expression is less influenced by population stratification than genetic association, the findings may still be biased due to different allele frequencies and LD structures in different ethnic groups. Correlation analysis of gene expression (*PGAP3, GSDMB*, and *GSDMA*) and asthma

phenotypes (Table IV) and eQTL analysis of top five eQTL SNPs for these three genes (Table E4) were also performed in SARP non-Hispanic Whites (Table E24 and Table E25). The findings of gene expression correlation and eQTL analyses were similar between non-Hispanic Whites and all subjects with mixed races. In summary, the association of SNPs in *GSDMB*, the expression levels of *GSDMB*, and asthma phenotypes make *GSDMB* a strong candidate for severe asthma.

The function of *PGAP3, GSDMB*, or *GSDMA* is not totally understood. *PGAP3* may have a role in controlling autoimmunity and Th1/Th2 balance.[90] *GSDMA* may regulate or be regulated by TGF-β1 and mediate immune defense by inducing pyroptosis.[91] *GSDMB* may regulate apoptosis of epithelial cells and upregulate expression of airway remodeling genes, chemokines, and heat-shock proteins.[89,91] In this study, the expression levels of *GSDMB* are positively correlated with MHC class I molecules (*HLA-A/-B/-C/-F*), type I interferon (*STAT1, STAT2*, and *IRF9*) and type II interferon pathway genes (*IFN-γ* and *STAT1*), and Th1 pathway genes (*IFN-γ, STAT1, IL18R1*, and *IL18BP*). All these biological pathways are related to antiviral process, indicating that virus infection and expression of antiviral pathway genes may lead to severe asthma and asthma exacerbations. rs7216389 in *GSDMB* has been associated with human rhinovirus (HRV) induced wheezing illnesses in children and increased expression of *GSDMB* and *ORMDL3* in HRV-stimulated peripheral-blood mononuclear cells, which further indicates the potential interaction of *GSDMB* and virus infection in asthma pathogenesis.[92] In a previous gene expression analysis in human nasal epithelial cells, *GSDMB* expression can be induced by IFN-α stimulation.[93] In this study, two SNPs (rs1031458 and rs3902920) in the promoter region of *GSDMB* are colocalized with IRF binding sites and associated with *GSDMB* expression, atopic early onset asthma, asthma severity, and longitudinal asthma exacerbations, making them potential functional SNPs.

One main disadvantage of this study is the relatively small sample size. In genetic association, eQTL, and gene expression correlation analyses, nominal p-values of 0.05 in addition to adjusted p-values have been used. Furthermore, the replication results in several datasets are not always consistently significant. Thus, it requires careful interpretation as for significance and replication. One main advantage of this study is that multi-level evidence point to the same gene (*GSDMB*).

In conclusion, we identified that three independent signals (*PGAP3, GSDMB*, and *GSDMA*) were associated with asthma susceptibility and *GSDMB* was also associated with asthma severity, asthma exacerbations, and antiviral pathways. Future candidate gene studies in large, multiethnic, or children with asthma and functional experiments may further reveal functional SNPs/genes for asthma including rare variants in this important region.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ACQ-6	asthma control questionnaire-6
AD	autoimmune diseases
BEC	bronchial epithelial cells
CD	Crohn's disease
eQTL	expression quantitative trait loci
ER	emergency room
FDR	false discovery rate
GSDMA	gasdermin A
GSDMB	gasdermin B
GTEx	Genotype-Tissue Expression database

GWAS	genome-wide association study
HRV	human rhinovirus
IBD	inflammatory bowel disease
LD	linkage disequilibrium
MAF	minor allele frequency
MS	multiple sclerosis
ORMDL3	ORMDL sphingolipid biosynthesis regulator 3
PGAP3	post-GPI attachment to proteins 3
PBCh	primary biliary cholangitis
PBCi	primary biliary cirrhosis
QC	quality control
RA	rheumatoid arthritis
RNAseq	RNA sequence
SARP	Severe Asthma Research Program
SLE	systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SS	systemic sclerosis
TOPMed	Trans-Omics for Precision Medicine
T1D	type I diabetes
UC	ulcerative colitis
WGS	whole-genome sequence

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Key Messages

- SNPs in *GSDMB* were associated with asthma, asthma severity, asthma exacerbations, and *GSDMB* expression levels, and its expression levels were correlated with asthma, asthma exacerbations, and antiviral pathways.
- SNPs in *PGAP3-ERBB2, ZPBP2-GSDMB-ORMDL3*, and *GSDMA* regions were associated with the expression levels of *PGAP3, GSDMB*, and *GSDMA*, respectively; SNPs in *IKZF3* were associated with the expression levels of *PGAP3, GSDMB*, or *GSDMA*.
- SNPs identified by GWAS of asthma or autoimmune diseases (AD) were also eQTL SNPs for *PGAP3, GSDMB*, or *GSDMA*, but showed opposite effect alleles between asthma and AD.

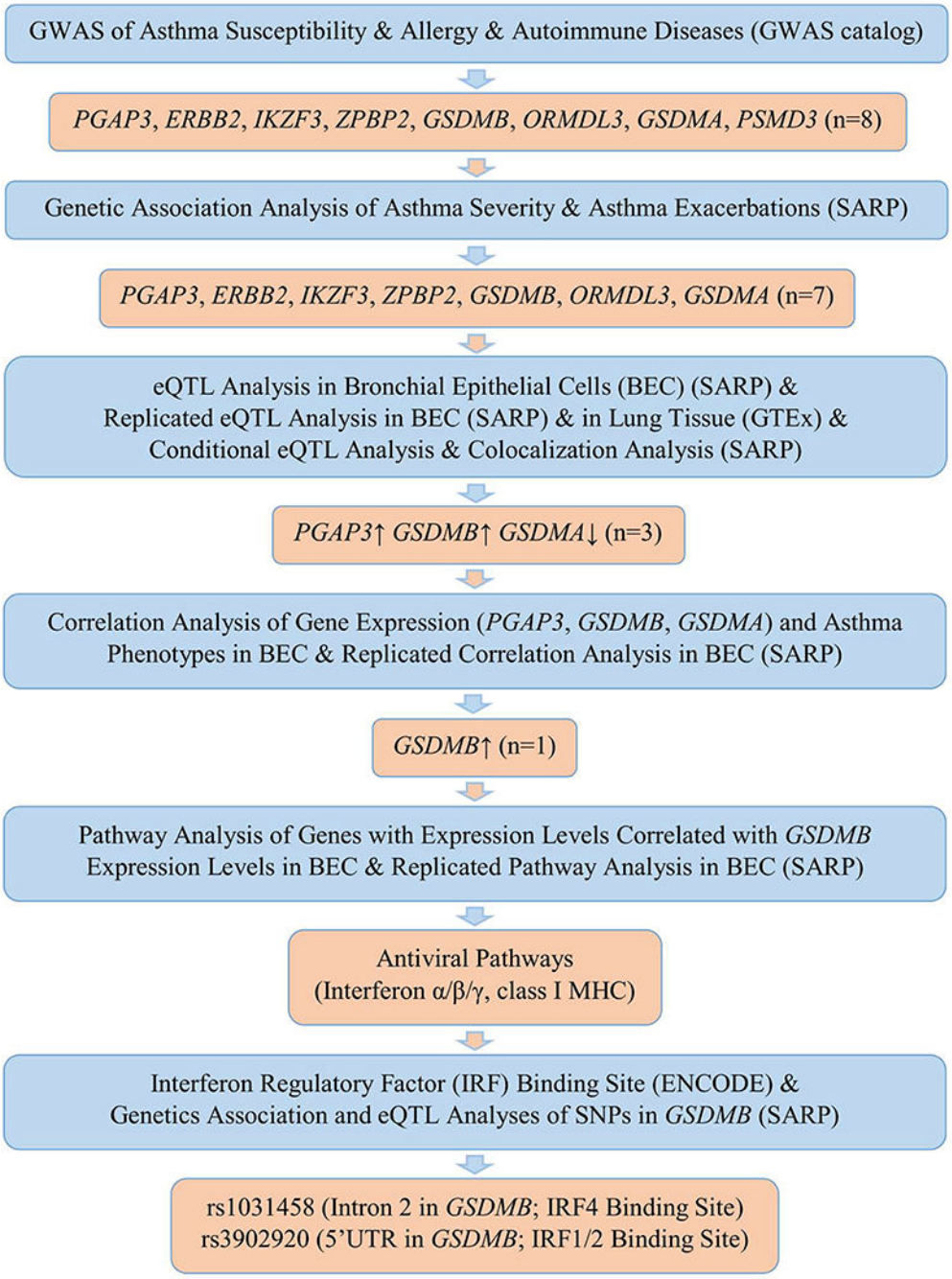

FIG 1.
Flow chart of genetic analyses in chr17q12-21.2 region

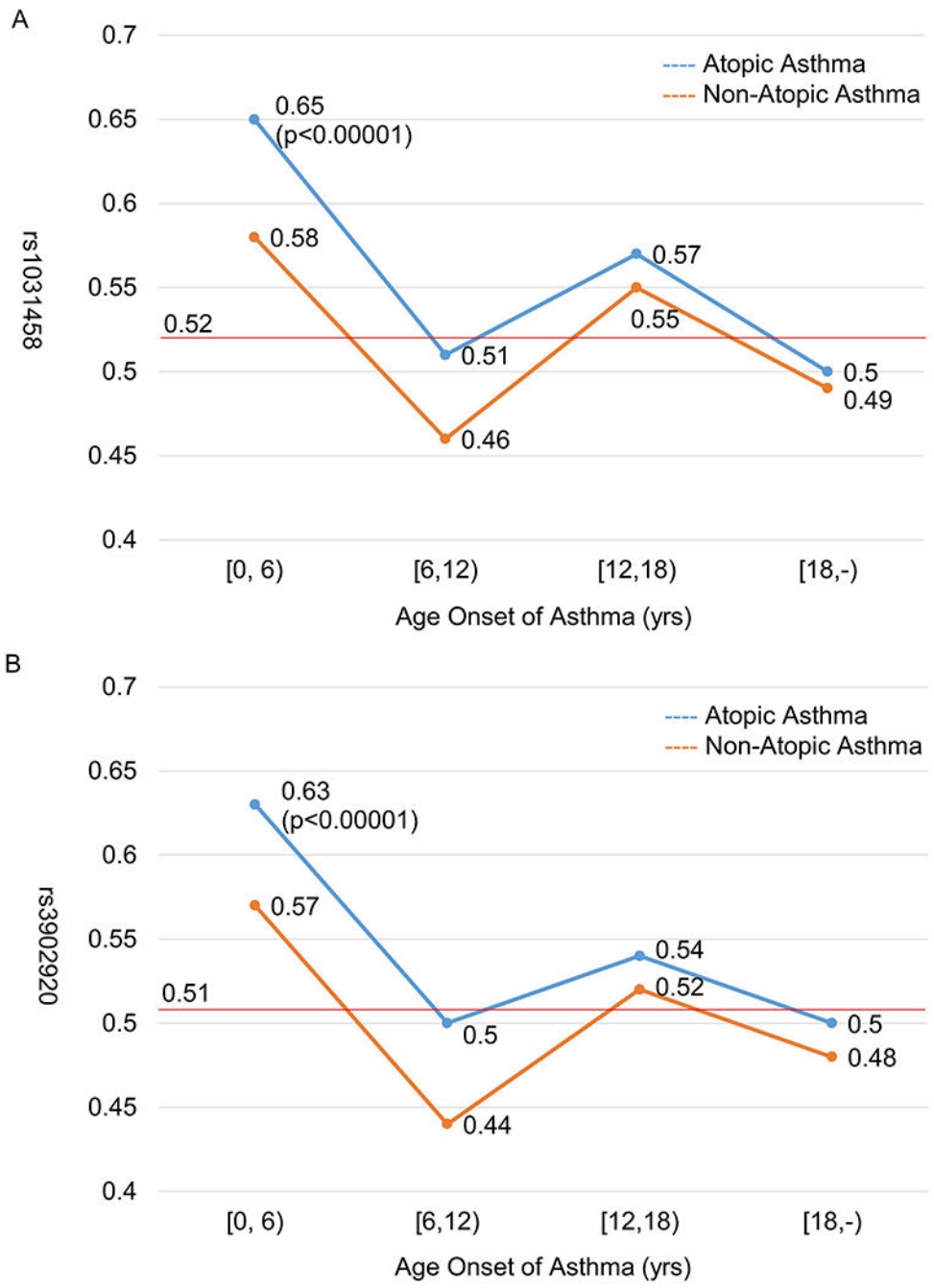

FIG 2.

Risk allele frequency of rs1031458 (A) and rs3902920 (B) in *GSDMB* stratified by age onset of asthma and atopic status. Chi-square test was performed by comparing each asthma group with general North-Western European controls shown in red line (gnomAD V2.1.1; https://gnomad.broadinstitute.org/).

TABLE I.

Demographics (mean±SDs) of subjects in SARP

	RNAseq (BEC) Longitudinal		Microarray (BEC) Cross-sectional		WGS†				
	All	WGS	All	GWAS	Severe Asthma	Non-severe Asthma	Longitudinal Exacerbations	Non-Hispanic White	African American
n	156	114	155	120	426	531	273	1,016	622
Age	41±13	41±13	37±13	36±13	46±15	37±15	47±16	39±17	29±17
Female, n (%)	99 (63)	74 (65)	101 (65)	80 (67)	269 (63)	353 (66)	176 (64)	656 (65)	369 (59)
BMI	30±8.1	30±8.7	30±6.8	30±7.0	31±7.7	28±7.6	31±7.9	29±7.9	30±11
Race (Non-Hispanic White/African American/Other*), %	67/24/9	65/26/9	62/29/9	60/29/11	100/0/0	100/0/0	100/0/0	100/0/0	0/100/0
Baseline % predicted FEV_1	82±21	76±20	76±22	76±23	66±22	84±17	73±21	77±21	78±20
Baseline FEV_1/FVC	0.73±0.10	0.70±0.10	0.72±0.12	0.71±0.13	0.66±0.13	0.75±0.09	0.69±0.11	0.72±0.12	0.72±0.11
Asthma status (Control/ Non-severe/Severe), n	42/49/65	0/49/65	27/78/50	19/60/41	0/0/426	0/531/0	0/111/162	0/564/451	0/270/252
Age Onset of Asthma<18 yrs, n (%)	77 (68)	77 (68)	88 (71)	69 (68)	238 (56)	348 (66)	164 (60)	645 (64)	394 (75)

Note: BEC: bronchial epithelial cell brushing; Microarray: Agilent Whole Human Genome Microarray 4x44K v2; WGS: whole-genome sequence; GWAS: genome-wide association study.

*Other races include Hispanic, Asian, American Indian, and mixed.

†1,016 non-Hispanic Whites and 622 African Americans in SARP longitudinal and cross-sectional cohorts with WGS were used for LD calculation; Among 1,016 non-Hispanic Whites, 957 adults (age ⩾ 12 yrs) (426 with severe asthma vs. 531 with non-severeasthma) were included in the genetic association analysis of asthma severity; 273 adults in the longitudinal cohort were included in the genetic association analysis of longitudinal asthma exacerbations.

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TABLE II.

Genetic association and eQTL results of 48 GWAS-identified SNPs associated with expression levels of *PGAP3, GSDMB*, or *GSDMA* in the longitudinal cohort

SNP	Position (hg38)	SNP Type	Gene	Allele‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
rs2941504*	39674647	synonymous	*PGAP3*	A/G	Asthma[2]; eQTL for PGAP3[3]	1	1	A	1.05	0.64	0.97	0.011	0.77 (5E-9)	0.30 (0.03)	−0.32 (0.02)	A ~ PGAP3↑ (2E-10); ORMDL3↑ (1E-7); GSDMA↓ (4E-6)
rs2517955*	39687428	intronic	*PGAP3*	C/T	eQTL for ORMDL3[4]			C	1.10	0.35	1.05	0.0034	0.56 (5E-6)	0.28 (0.03)	−0.11 (0.4)	C ~ PGAP3↑ (1E-12); ORMDL3↑ (2E-6)
rs2952156*†	39720582	intronic	*ERBB2*	A/G	Asthma[5]	2		A	1.06	0.56	0.99	0.0081	0.76 (4E-8)	0.30 (0.04)	−0.33 (0.02)	A ~ PGAP3↑ (4E-10); ORMDL3↑ (4E-9); GSDMA↓ (7E-6); GSDMB↑ (2E-5)
rs4252665†	39729130	intronic	*ERBB2*	T/C	SLE[31]			T	0.69	0.15	−0.40	0.69	−0.02 (1.0)	0.04 (0.9)	−0.28 (0.5)	NS
rs2941522†	39754115	intergenic	*GRB7-IKZF3*	T/C	Asthma[6]			T	1.24	0.028	0.84	0.019	0.45 (8E-4)	0.33 (0.02)	−0.54 (6E-5)	T ~ GSDMB↑ (3E-15); ORMDL3↑ (4E-15); GSDMA↓ (1E-6); PGAP3↑ (1E-6)
rs12946510†	39756124	integenic	*GRB7-IKZF3*	T/C	UC[32–33]; CD[32–33];		2	T	0.86	0.12	−0.72	0.046	−0.43 (0.002)	−0.43 (0.002)	0.44 (0.002)	T ~ GSDMB↓ (2E-9); ORMDL3↓

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
					MS[34]; IBD[32–33,35]											(9E-9); GSDMA↑ (1E-6)
rs2941509†	39764941	3′ UTR	*IKZF3*	A/G	SLE[36–37]			A	0.81	0.44	0.46	0.65	−0.08 (0.7)	0.04 (0.9)	0.02 (0.9)	A ~ GSDMB↓ (3E-5)
rs907092*†	39766006	synonymous	*IKZF3*	G/A A/G	Asthma[7–8]; PBCh[38]			G	1.24	0.027	0.66	0.069	0.47 (8E-4)	0.50 (4E-4)	−0.43 (0.003)	G ~ GSDMB↑ (2E-9); ORMDL3↑ (5E-9); GSDMA↓ (2E-7)
rs10445308†	39781794	intronic	*IKZF3*	C/T	Atopy[27]			C	1.23	0.033	0.76	0.034	0.47 (7E-4)	0.51 (2E-4)	−0.45 (1E-3)	C ~ GSDMB↑ (7E-10); ORMDL3↑ (3E-9); GSDMA↓ (3E-8)
rs12450323†	39816455	intronic	*IKZF3*	T/G	Asthma[9]			T	1.15	0.24	0.96	0.023	0.63 (2E-4)	0.43 (0.01)	0.13 (0.4)	T ~ ORMDL3↑ (1E-5); GSDMB↑ (2E-5); PGAP3↑ (2E-5)
rs9303277*†	39820216	intronic	*IKZF3*	T/C	PBCh[39–42]; SS[43]; SLE[43]			T	0.79	0.017	−0.84	0.017	−0.48 (4E-4)	−0.40 (0.004)	0.49 (3E-4)	T ~ GSDMB↓ (7E-15); ORMDL3↓ (6E-14); GSDMA↑ (2E-7); PGAP3↓ (7E-6)
rs143123127†	39850937	intronic	*IKZF3*	A/G	SLE[37]			A	0.80	0.43	−0.38	0.73	−0.02 (1.0)	0.30 (0.5)	−0.77 (0.06)	NA
rs9635726†	39863888	intronic	*IKZF3*	C/T	PBCh[44]			C	0.88	0.27	−1.14	0.0078	−0.63 (1E-4)	−0.47 (0.005)	−0.07 (0.7)	C ~ GSDMB↓ (1E-5);

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
																ORMDL3↓ (2E-5); PGAP3↓ (2E-5)
rs4795397†	39867492	intergenic	*IKZF3-ZPBP2*	A/G (G/A)	Asthma[8] IBD[33]			A	1.17	0.027	0.81	0.022	0.40 (0.003)	0.52 (1E-4)	−0.42 (0.002)	A ~ ORMDL3↑ (4E-9); GSDMA↓ (2E-8); GSDMB↑ (7E-8)
rs11655198†	39869916	intronic	*ZPBP2*	(C/T)	Asthma[10]			C	1.29	0.0097	0.82	0.019	0.40 (0.003)	0.44 (0.001)	−0.30 (0.03)	C ~ GSDMB↑ (6E-15); ORMDL3↑ (8E-12); GSDMA↓ (8E-7); PGAP3↑ (3E-6)
rs12936231*	39872867	intronic	*ZPBP2*	(C/G)	ORMDL3 promoter[11]		3	C	1.29	0.0089	0.87	0.013	0.40 (0.004)	0.42 (0.002)	−0.37 (0.008)	C ~ GSDMB↑ (5E-15); ORMDL3↑ (1E-14); GSDMA↓ (1E-7); PGAP3↑ (8E-6)
rs59716545†	39875604	intronic	*ZPBP2*	(G/T)	RA[45]			G	0.82	0.042	−0.89	0.012	−0.38 (0.005)	−0.52 (1E-4)	0.31 (0.02)	NA
rs12939457†	39875935	intronic	*ZPBP2*	T/C	Allergic rhinitis[28]			T	1.21	0.054	0.90	0.011	0.44 (0.002)	0.50 (3E-4)	−0.40 (0.004)	NA
rs35736272†	39876427	intronic	*ZPBP2*	(C/T)	AD[46]			C	0.81	0.033	−0.89	0.012	−0.40 (0.003)	−0.53 (6E-5)	0.35 (0.01)	C ~ ORMDL3↓ (7E-10); GSDMB↓ (3E-9); GSDMA↑ (4E-9);

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
rs12232497†	39883866	intergenic	*ZPBP2-GSDMB*	C/T	AD[47]			C	0.81	0.033	−0.89	0.012	−0.40 (0.003)	−0.53 (6E-5)	0.35 (0.01)	C ~ ORMDL3↓ (7E-10); GSDMA↑ (3E-9); GSDMB↓ (3E-9)
rs2872507†	39884510	intergenic	*ZPBP2-GSDMB*	A/G	RA[48–49]; T1D[50]; UC[51]; CD[52–53]			A	0.81	0.035	−0.89	0.012	−0.42 (0.002)	−0.51 (2E-4)	0.35 (0.01)	A ~ ORMDL31 (3E-10); GSDMB 1 (5E-10); GSDMA1 (2E-9)
rs12936409†	39887396	intergenic	*ZPBP2-GSDMB*	T/C	RA[48,54]			T	0.81	0.030	−0.88	0.014	−0.43 (0.002)	−0.52 (1E-4)	0.36 (0.009)	T ~ ORMDL3↓ (8E-10); GSDMA↑ (4E-9); GSDMB↓ (9E-9)
rs8067378†	39895095	intergenic	*ZPBP2-GSDMB*	G/A	PBCi[55]			G	0.76	0.0054	−0.88	0.011	−0.43 (0.002)	−0.42 (0.003)	0.36 (0.01)	G ~ GSDMB↓ (5E-15); ORMDL3↓ (1E-14); GSDMA↑ (1E-7); PGAP3↓ (8E-6)
rs12453507†	39896954	intergenic	*ZPBP2-GSDMB*	(G/C)	T1D[56]			G	0.76	0.0050	−0.78	0.024	−0.36 (0.007)	−0.43 (1E-3)	0.29 (0.03)	G ~ GSDMB↓ (2E-15); ORMDL3↓ (2E-12); GSDMA↑ (2E-8); PGAP3↓ (1E-6)
rs8069176*†	39900944	intergenic	*ZPBP2-GSDMB*	G/A	Asthma[5]			G	1.26	0.020	0.86	0.015	0.39 (0.003)	0.57 (9E-6)	−0.33 (0.01)	G ~ ORMDL3↑ (6E-11); GSDMA↓

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
																(1E-10); GSDMB↑ (2E-10)
rs4795399†	39905186	intronic	*GSDMB*	T/C	Asthma[12†13]			T	1.26	0.019	0.88	0.013	0.35 (0.007)	0.55 (2E-5)	−0.31 (0.02)	T ~ ORMDL3↑ (1E-10); GSDMA↓ (2E-10); GSDMB↑ (7E-10)
rs2305480*†	39905943	missense	*GSDMB*	G/A A/G	Asthma[14–15] RA[48]; UC[57]			G	1.27	0.015	0.93	0.0086	0.35 (0.007)	0.55 (3E-5)	−0.31 (0.02)	G ~ ORMDL3↑ (1E-10); GSDMA↓ (2E-10); GSDMB↑ (2E-9)
rs2305479†	39905964	missense	*GSDMB*	(C/T)	Asthma[5]			C	1.34	0.0029	0.85	0.014	0.33 (0.01)	0.46 (3E-4)	−0.26 (0.05)	C ~ GSDMB↑ (6E-15); ORMDL3↑ (4E-15); GSDMA↓ (1E-7); PGAP3↑ (5E-6)
rs62067034†	39907485	intronic	*GSDMB*	(C/T)	Asthma[16]			C	1.34	0.0029	0.85	0.014	0.33 (0.01)	0.46 (3E-4)	−0.26 (0.05)	C ~ GSDMB↑ (2E-15); ORMDL3↑ (1E-12); GSDMA↓ (1E-7); PGAP3↑ (7E-6)
rs11078927*†	39908152	intronic	*GSDMB*	C/T	Asthma[8,17]			C	1.26	0.018	0.95	0.0073	0.37 (0.005)	0.56 (2E-5)	−0.35 (0.009)	C ~ ORMDL3↑ (1E-10); GSDMA↓ (3E-10); GSDMB↑ (9E-10)

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
rs11078928*	39908216	receptor	*GSDMB*	T/C	eQTL for GSDMB[18]			T	1.26	0.018	0.95	0.0073	0.37 (0.005)	0.56 (2E-5)	−0.35 (0.009)	T ~ ORMDL3↑ (8E-11); GSDMA↓ (4E-10); GSDMB↑ (8E-10)
rs117097909†	39908718	intronic	*GSDMB*	A/G	Asthma[13]			A	1.04	0.860	−0.04	0.95	0.27 (0.37)	0.52 (0.09)	0.21 (0.49)	NA
rs2290400*†	39909987	intronic	*GSDMB*	T/C (C/T)	Asthma[19] T1D[58]	3	4	T	1.32	0.0044	0.84	0.016	0.40 (0.003)	0.41 (0.003)	−0.35 (0.01)	T ~ GSDMB↑ (8E-16); ORMDL3↑ (7E-15); GSDMA↓ (1E-8); PGAP3↑ (3E-6)
rs4795400†	39910767	intronic	*GSDMB*	(C/T)	Allergy[10]			C	1.27	0.015	0.91	0.0093	0.35 (0.007)	0.55 (3E-5)	−0.31 (0.02)	C ~ ORMDL3↑ (2E-10); GSDMB↑ (3E-10); GSDMA↓ (5E-10)
rs869402†	39911790	intronic	*GSDMB*	(C/T)	Asthma[20]			C	1.33	0.0039	0.81	0.019	0.31 (0.01)	0.41 (1E-3)	−0.24 (0.06)	C ~ GSDMB↑ (1E-15); ORMDL3↑ (2E-12); GSDMA↓ (2E-7); PGAP3↑ (3E-6)
rs921650†	39912823	intronic	*GSDMB*	A/G	Allergy[29]			A	1.32	0.0045	0.80	0.021	0.33 (0.009)	0.47 (2E-4)	−0.27 (0.03)	A ~ GSDMB↑ (1E-15); ORMDL3↑ (8E-13); GSDMA↓ (2E-7);

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
																PGAP3↑ (3E-6)
rs7216389*†	39913696	intronic	*GSDMB*	T/C	Asthma[1]			T	1.32	0.0045	0.80	0.021	0.33 (0.009)	0.47 (2E-4)	−0.27 (0.03)	T ~ GSDMB↑ (1E-15); ORMDL3↑ (8E-13); GSDMA↓ (2E-7); PGAP3↑ (3E-6)
rs9303280†	39917778	intronic	*GSDMB*	C/T	Allergy[30]			C	1.32	0.0049	0.77	0.028	0.37 (0.004)	0.49 (1E-4)	−0.33 (0.01)	C ~ GSDMB↑ (3E-15); ORMDL3↑ (2E-12); GSDMA↓ (3E-9); PGAP3↑ (9E-6)
rs4065275*	39924612	intronic	*ORMDL3*	G/A	ORMDL3 promoter[11]			G	1.31	0.0064	0.82	0.020	0.31 (0.02)	0.36 (0.008)	−0.29 (0.04)	G ~ GSDMB↑ (1E-13); ORMDL3↑ (1E-13); GSDMA↓ (6E-11)
rs8076131*	39924659	intronic	*ORMDL3*	A/G	Early wheeze[21]			A	1.25	0.022	0.85	0.015	0.36 (0.007)	0.47 (3E-4)	−0.37 (0.006)	A ~ GSDMA↓ (1E-11); ORMDL3↑ (7E-11); GSDMB↑ (1E-9)
rs12603332*	39926554	5′ UTR	*ORMDL3*	C/T	eQTL and meQTL for O RMD L3/ GSDMB[22–23]	4	5	C	1.31	0.0060	0.83	0.018	0.29 (0.03)	0.40 (0.003)	−0.44 (9E-4)	C ~ ORMDL3↑ (2E-14); GSDMB↑ (2E-13); GSDMA↓ (6E-12); PGAP3↑ (2E-5)

SNP	Position (hg38)	SNP Type	Gene	Allelet‡	Associated Trait**	LD†† (NHW)	LD†† (AA)	Asthma Severity***			Number of Exacerbations in 3 years†††		eQTL of SARP3 in BEC (n=114)			eQTL of GTEx database Lung Tissue (n=383)
								Effect Allele	OR	P	β	P	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
rs4794820†	39933091	intronic	*ORMDL3*	(G/A)	Asthma[24]			G	1.26	0.021	0.94	0.0077	0.28 (0.04)	0.40 (0.002)	−0.38 (0.004)	G ~ GSDMA↓ (2E-17); ORMDL3↑ (2E-11); GSDMB↑ (2E-8)
rs6503525†	39938921	intergenic	*ORMDL3-LRRC3C*	(C/G)	Asthma[25]	5		C	1.23	0.038	0.57	0.12	0.25 (0.06)	0.076 (0.6)	−0.37 (0.005)	C ~ GSDMA↓ (6E-21); GSDMB↑ (9E-8); ORMDL3↑ (2E-5)
rs3902025†	39963001	5′ UTR	*GSDMA*	C/A	SS[59]		6	C	0.81	0.033	−0.65	0.072	−0.30 (0.02)	−0.23 (0.09)	0.48 (3E-4)	C ~ GSDMA↑ (2E-21); ORMDL3↓ (9E-7); GSDMB↓ (1E-5)
rs3894194*†	39965740	missense	*GSDMA*	A/G G/A	Asthma[14–15] SS[60]			A	1.14	0.20	0.33	0.35	0.36 (0.006)	0.10 (0.4)	−0.46 (4E-4)	A ~ GSDMA↓ (1E-21); GSDMB↑ (4E-9); ORMDL3↑ (2E-7)
rs7212938†	39966427	missense	*GSDMA*	G/T	Asthma[9]			G	1.14	0.19	0.28	0.44	0.27 (0.04)	0.14 (0.3)	−0.28 (0.03)	G ~ GSDMA↓ (3E-18); GSDMB↑ (1E-7); ORMDL3↑ (5E-5)
rs3859192*	39972395	intronic	*GSDMA*	T/C	eQTL for GSDMA[26]			T	0.96	0.68	0.44	0.23	0.41 (0.002)	0.19 (0.2)	−0.42 (0.001)	T ~ GSDMA↓ (5E-52)
rs11652139†	39992780	intronic	*PSMD3*	(A/G)	Allergy[29]			A	1.01	0.90	0.47	0.19	0.32 (0.02)	0.29 (0.03)	−0.17 (0.2)	NA

Note: entries with p-value<0.0035 for genetic association analysis of asthma severity or longitudinal asthma exacerbations were labeled in red color. NS: non-significant; NA: non-available.

[*] 17 SNPs associated with asthma and reported by Stein et al.[62]

[†] SNPs associated with asthma, allergy, and autoimmune diseases from NHGRI-EBI GWAS catalog (www.ebi.ac.uk/gwas/),[78] incorporated in UCSC genome browser (genome.ucsc.edu; accessed on March 1, 2019).[79]

[‡] Risk allele/Other allele: parenthesis indicates risk allele was not reported in the original study but predicted based on available data.

[**] AD: autoimmune diseases; CD: Crohn's disease; IBD: inflammatory bowel disease; MS: multiple sclerosis; PBCh: primary biliary cholangitis; PBCi: primary biliary cirrhosis; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SS: systemic sclerosis; T1D: type I diabetes; UC: ulcerative colitis.

[††] LD was estimated with 95% confidence intervals of D' to define LD blocks of 48 SNPs for 1,016 non-Hispanic Whites (NHW) and 622 African Americans (AA) in SARP longitudinal cohort and cross-sectional cohort with WGS using Haploview.[81]

[***] OR and P were odds ratio and p-value for genetic association analysis of asthma severity (426 severe vs. 531 non-severe asthma) in non-Hispanic White in SARP.

[†††] β and P were correlation coefficient and p-value for genetic association analysis of the number of longitudinal exacerbations due to asthma in 3 years in the longitudinal cohort in 273 asthmatics with longitudinal asthma exacerbations in non-Hispanic White in SARP.

TABLE III.

eQTL results of 26 SNPs significantly associated with expression levels of *PGAP3*, *GSDMB*, or *GSDMA* in the longitudinal cohort

SNP	Position (hg38)	Gene	LD* (NHW)	LD* (AA)	SARP3 BEC (n=114)						eQTL of GTEx database Lung Tissue (n=383)†
					A1	PGAP3		GSDMB		GSDMA		
						β	P	β	P	β	P	
rs3751903	39627534	*PPP1R1B*	1	1	C	0.43	0.002	0.55	1.0E-4	−0.10	0.5	C ~ PGAP3↑ (1E-7)
rs3794712	39635234	*PPP1R1B*			A	0.48	0.007	0.78	6.5E-6	0.06	0.7	NS
rs10558975	39675051	*PGAP3*			G	−0.54	1.1E-5	−0.32	0.01	0.13	0.3	G ~ PGAP3↓ (1E-13); ORMDL3↓ (2E-5); GSDMB↓ (6E-5)
rs907088	39677314	*PGAP3*		2	G	0.69	3.3E-7	0.32	0.02	−0.27	0.06	G ~ PGAP3↑ (1E-12); ORMDL3↑ (8E-8)
rs2517954	39687297	*PGAP3*			T	0.77	3.1E-9	0.25	0.08	−0.29	0.04	T ~ PGAP3↑ (9E-10); ORMDL3↑ (3E-8)
rs2904765	39692422	*PGAP3-ERBB2*			T	0.70	8.7E-6	0.17	0.3	−0.34	0.04	NS
rs56328874	39694273	*PGAP3-ERBB2*			A	−0.72	1.0E-4	0.16	0.4	0.26	0.2	NS
rs2517951	39696844	*PGAP3-ERBB2*	2	3	T	−0.58	1.6E-6	−0.29	0.02	0.13	0.3	T ~ PGAP3↓ (1E-13); ORMDL3↓ (2E-6); GSDMB↓ (2E-5)
rs2952155	39705465	*ERBB2*			T	0.72	4.6E-7	0.31	0.04	−0.23	0.1	T ~ PGAP3↑ (2E-8); ORMDL3↑ (1E-6)
rs2934967	39714125	*ERBB2*			G	0.79	9.9E-9	0.25	0.08	−0.29	0.05	G ~ PGAP3↑ (5E-11); ORMDL3↑ (2E-8); GSDMB↑ (4E-5); GSDMA↓ (6E-6)
rs2941520	39747477	*GRB-IKZF3*		4	T	0.70	1.7E-6	0.30	0.05	0.01	0.9	T ~ PGAP3↑ (2E-10); ORMDL3↑ (3E-9); GSDMB↑ (1E-6)
rs2941519	39747478	*GRB-IKZF3*			G	−0.36	0.01	−0.15	0.3	0.52	1.6E-4	G ~ ORMDL3↓ (5E-14); GSDMB↓ (6E-13); PGAP3↓ (5E-6); GSDMA↑ (3E-5)
rs9747973	39748854	*GRB-IKZF3*			C	−0.41	0.003	−0.34	0.01	0.53	1.0E-4	C ~ GSDMB↓ (2E-15); ORMDL3↓ (7E-15); GSDMA↑ (6E-7); PGAP3↓ (1E-6)
rs12450323	39816455	*IKZF3*			T	0.63	1.5E-4	0.43	0.01	0.13	0.4	T ~ ORMDL3↑ (1E-5); GSDMB↑ (2E-5); PGAP3↑ (2E-5)
rs114211283	39819840	*IKZF3*			A	1.11	3.5E-5	0.13	0.6	0.12	0.7	NS
rs62066988	39836028	*IKZF3*			T	−0.35	0.02	−0.56	1.4E-4	0.31	0.04	T ~ ORMDL3↓ (2E-6); GSDMB↓ (2E-5); GSDMA↑ (4E-5)
rs9635726	39863888	*IKZF3*	3		T	0.63	1.3E-4	0.47	0.005	0.07	0.7	T ~ GSDMB↑ (1E-5); ORMDL3↑ (2E-5); PGAP3↓ (2E-5)
rs4795397	39867492	*IKZF3-ZPBP2*		5	G	−0.40	0.003	−0.52	1.0E-4	0.42	0.002	G ~ ORMDL3↓ (4E-9); GSDMA↑ (2E-8); GSDMB↓ (7E-8)
rs12150079	39869164	*ZPBP2*			A	−0.38	0.01	−0.59	5.7E-5	0.32	0.03	A ~ ORMDL3↓ (3E-7); GSDMB↓ (6E-6); GSDMA↑ (7E-6)

SNP	Position (hg38)	Gene	LD* (NHW)	LD* (AA)	SARP3 BEC (n=114)						eQTL of GTEx database Lung Tissue (n=383)†
					A1	PGAP3		GSDMB		GSDMA		
						β	P	β	P	β	P	
rs11651596	39899863	*ZPBP2-GSDMB*		6	C	−0.36	0.008	−0.62	1.6E-6	0.32	0.02	C ~ ORMDL3↓ (2E-10); GSDMA↑ (3E-10); GSDMB↓ (2E-9)
rs11657449	39901588	*ZPBP2-GSDMB*			C	−0.34	0.02	−0.69	7.5E-7	0.32	0.03	C ~ ORMDL3↓ (2E-7); GSDMA↑ (7E-7); GSDMB↓ (6E-6)
rs1011082	39912261	*GSDMB*			T	−0.33	0.01	−0.51	6.6E-5	0.31	0.02	T ~ GSDMB↓ (8E-16); ORMDL3↓ (6E-13); GSDMA↑ (1E-7); PGAP3↓ (4E-7)
rs201413617	39917590	*GSDMB-ORMDL3*			G	−0.33	0.01	−0.48	1.6E-4	0.27	0.04	NS
rs4795405	39932164	*ORMDL3-LRRC3C*			T	−0.35	0.01	−0.51	1.6E-4	0.45	0.0009	T ~ GSDMA↑ (3E-14); ORMDL3↓ (1E-10); GSDMB↓ (p=6E-10)
rs9914973	39966455	*GSDMA*			C	−0.42	0.002	−0.22	0.1	0.52	1.7E-4	C ~ GSDMA↑ (9E-17)
rs3859193	39969603	*GSDMA*			A	0.31	0.02	0.13	0.3	−0.50	7.2E-5	A ~ GSDMA↓ (9E-30); GSDMB↑ (5E-7)

Note: entries with p-value<1.98×10^{-4} were labeled in red color. β and P were correlation coefficient and p-value of eQTL analysis. BEC: bronchial epithelial cells brushing. NS: non-significant.

*LD was estimated with 95% confidence intervals of D' to define LD blocks of 26 SNPs for 1,016 non-Hispanic Whites (NHW) and 622 African Americans (AA) in SARP longitudinal cohort and cross-sectional cohort with WGS using Haploview.[81]

†eQTL of GTEx database: eQTL SNPs identified in the lung tissue (n=383) from Genotype-Tissue Expression (GTEx) database.[26] ↑indicated up-regulation of gene expression and ↓indicated down-regulation of gene expression.

TABLE IV.

Correlation of the expression levels of *PGAP3*, *GSDMB*, or *GSDMA* and asthma phenotypes in SARP

RNAseq (156 BEC) in the longitudinal cohort

Gene	Asthma Susceptibility			Asthma Severity			Number of Exacerbations (last 12 months)		ΔACQ6 (After-Before steroid trt)	
	Healthy Controls (n=42)	Asthma (n=114)	P value	Non-severe Asthma (n=49)	Severe Asthma (n=65)	P value	Correlation Coefficient (β)	P value (n=114)	Correlation Coefficient (β)	P value (n=109)
PGAP3	8.81±0.22	8.88±0.20	0.08	8.90±0.21	8.87±0.20	0.33	−0.84	0.57	−0.12	0.77
GSDMB	9.94±0.25	10.1±0.32	0.05	10.1±0.28	10.1±0.35	0.89	2.11	0.019	−0.81	0.0008
GSDMA	0.50±0.15	0.55±0.22	0.26	0.52±0.19	0.57±0.24	0.29	−1.40	0.27	0.14	0.69

Microarray (155 BEC) in the cross-sectional cohort

Gene	Asthma Susceptibility			Asthma Severity			ER or Hospitalization (last 12 months)		
	Healthy Controls (n=27)	Asthma (n=128)	P value	Non-severe Asthma (n=78)	Severe Asthma (n=50)	P value	No (n=77)	Yes (n=47)	P value
PGAP3	10.8±0.22	10.8±0.33	0.43	10.8±0.34	10.8±0.31	0.63	10.8±0.35	10.8±0.29	0.57
GSDMB	10.5±0.42	10.4±0.47	0.15	10.4±0.45	10.4±0.51	0.23	10.3±0.46	10.5±0.49	0.03
GSDMA	6.26±0.15	6.21±0.13	0.02	6.21±0.14	6.21±0.12	0.56	6.21±0.13	6.20±0.13	0.41

Note: a general linear model was used to test the correlation between gene expression levels (natural logarithm transformed in the longitudinal cohort or log2 transformed in the cross-sectional cohort) and asthma phenotypes with adjustment of age, sex, race, BMI, and batch effect.

Table V.

Biological pathways enriched for genes with expression levels correlated with *GSDMB* expression in BEC in the longitudinal cohort

Pathway	Gene	P value (FDR)
Interferon gamma signaling	CIITA, HLA-A, HLA-B, HLA-C, HLA-F, IFNG, IFNGR2, IL20RA, IRF9, MID1, OAS2, OAS3, PML, SP110, SP140L, STAT 1, SUMO1, TRIM14, TRIM22, TRIM25, TRIM26, TRIM38	2.3E-14
Interferon alpha/beta signaling	BST2, HLA-A, HLA-B, HLA-C, HLA-F, IFI27, IRF9, MX2, OAS2, OAS3, STAT1, STAT2, USP18, XAF1	2.3E-14
Antigen Presentation (Folding, assembly and peptide loading of class I MHC)	HLA-A, HLA-B, HLA-C, HLA-F, SEC24B, TAP1, TAP2, TAPBP	2.3E-14
ER-Phagosome pathway	HLA-A, HLA-B, HLA-C, HLA-F, IKBKB, PSMB9, PSMD8, PSME2, RAPSN, TAP1, TAP2, TAPBP	2.3E-14
Interferon Signaling	BST2, CIITA, HLA-F, IFI27, IFNG, IFNGR2, IL20RA, IRF9, MAPK1, MID1, MX2, NUP210, OAS2, OAS3, PML, SP110, SP140L, STAT1, STAT2, SUMO1, TRIM14, TRIM22, TRIM25, TRIM26, TRIM38, UBA7, USP18, XAF1	2.3E-14
Endosomal/Vacuolar pathway	HLA-A, HLA-B, HLA-C, HLA-F	2.3E-14
Antigen processing-Cross presentation	HLA-A, HLA-B, HLA-C, HLA-F, IKBKB, ITGB5, PSMB9, PSMD8, PSME2, RAPSN, TAP1, TAP2, TAPBP	2.3E-14
Class I MHC mediated antigen processing & presentation	ASB14, ASB4, ASB8, FBXL3, FBXO2, FBXO21, FBXO41, HLA-A, HLA-B, HLA-C, HLA-F, IKBKB, ITGB5, NARF, PJA2, POLL, PSMB9, PSMD8, PSME2, RAPSN, RBX1, RNF213, SEC24B, SIAH1, SKP1, TAP1, TAP2, TAPBP, TRIM36, TRIM39, UBA7, UBE2D2, UBE2D4, UBE2E3	3.5E-13
Immunoregulatory interactions (between a Lymphoid and a non-Lymphoid cell)	CD226, CLEC2D, HLA-A, HLA-B, HLA-C, HLA-F, RAET1E	6.3E-11
Cytokine Signaling in Immune system	ATF1, ATF2, CALM2, CIITA, CRKL, DUSP16, FASLG, HLA-A, HLA-B, HLA-C, HLA-F, IFI27, IKBKB, IL18BP, IL20RA, IL37, IL6ST, IRF9, LAMTOR3, LGALS9, LIFR, MID1, MX2, NDN, PDGFA, PML, PSME2, PTPN14, PTPN4, RBX1, SKP1, SP110, STAT1, STAT2, STAT6, TRIM14, TRIM22, TRIM25, TRIM26, TRIM38	7.9E-08
Adaptive Immune System	ACTR10, ASB4, ASB8, ASB14, BLNK, BTF3, BTN2A1, BTN2A2, BTN3A1, CALM2, CARD11, CD74, CLEC2D, DCTN3, DCTN6, FBXL3, FBXO41, GRAP2, HLA-A, HLA-B, HLA-C, IKBKB, MAP3K14, POLL, PSMD8, RAET1E, SEC24B, SIAH1, SKP1, TAP1, TAP2, TBCB, TEP1, TRIM36, TRIM39, UBA7, UBE2D2, UBE2D4, UBE2E3, ZAP70	9.7E-03

Note: pathways with false discovery rate (FDR)-adjusted p-value less than 0.05 were included.

TABLE VI.

Genetic association and eQTL results of 6 SNPs in IRF binding site of *GSDMB* in SARP.

SNP	Position (hg38)	SNP Type	IRF Binding Sites	Allele* (MAF)	Asthma Severity†	Number of Exacerbations in 3 years‡	eQTL in BEC (n=114) Longitudinal Cohort**			eQTL GTEx database Lung Tissue (n=383)††
					OR (P)	β (P)	PGAP3 β (P)	GSDMB β (P)	GSDMA β (P)	
rs1031458	39915920	intronic	IRF4	G/T (0.45)	0.76 (0.0053)	−0.77 (0.028)	−0.36 (5.1E-3)	−0.47 (2.4E-4)	0.31 (0.018)	GSDMB↓ (1E-20) ORMDL3↓ (3E-19) GSDMA↑ (1E-14) PGAP3↓ (1E-7)
rs3902920	39918763	5′UTR	IRF1/2	T/C (0.46)	0.75 (0.0027)	−0.88 (0.012)	−0.29 (0.036)	−0.45 (0.0011)	0.27 (0.047)	NA
rs77929191	39915767	intronic	IRF4	A/G (0.0021)	0.37 (0.40)	−2.9 (0.47)	−0.44 (0.54)	−1.26 (0.078)	−0.045 (0.95)	NS
rs536439445	39918670	5′UTR	IRF1/2	A/G (0.0052)	0.57 (0.41)	−1.8 (0.53)	0.77 (0.45)	−1.40 (0.17)	0.011 (0.99)	NS
rs549170154	39918764	5′UTR	IRF1/2	C/T (0.0021)	0.68 (0.74)	5.1 (0.21)	0.20 (0.78)	−0.18 (0.80)	−0.012 (0.99)	NA
rs540139228	39918797	5′UTR	IRF1/2	G/A (0.0010)	1.3 (0.86)	−2.6 (0.52)	NA	NA	NA	NA

*Minor allele (effect allele)/major allele (minor allele frequency).

†OR and P were odds ratio and p-value for genetic association analysis of asthma severity (426 severe vs. 531 non-severe asthma) in non-Hispanic White in SARP.

‡β and P were correlation coefficient and p-value for genetic association analysis of the number of longitudinal exacerbations due to asthma in 3 years in the longitudinal cohort in 273 asthmatics with longitudinal asthma exacerbations in non-Hispanic White in SARP.

**β and P were correlation coefficient and p-value for eQTL analysis in 114 subjects with RNAseq of bronchial epithelial cells in SARP longitudinal cohort.

††eQTL of GTEx database: eQTL SNPs identified in the lung tissue (n=383) from Genotype-Tissue Expression (GTEx) database.[26] ↑indicated up-regulation of gene expression and ↓indicated down-regulation of gene expression. NA: non-avaliable and NS: non-significant.