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Genome-wide association study identifies T_H1 pathway genes associated with lung function in asthmatic patients

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Abstract

Background—Recent meta-analyses of genome-wide association studies in general populations of European descent have identified 28 loci for lung function.

Objective—We sought to identify novel lung function loci specifically for asthma and to confirm lung function loci identified in general populations.

Methods—Genome-wide association studies of lung function (percent predicted FEV_1 [$ppFEV_1$], percent predicted forced vital capacity, and FEV_1/forced vital capacity ratio) were performed in 4 white populations of European descent (n = 1544), followed by meta-analyses.

Results—Seven of 28 previously identified lung function loci (*HHIP, FAM13A, THSD4, GSTCD, NOTCH4-AGER, RARB*, and *ZNF323*) identified in general populations were confirmed at single nucleotide polymorphism (SNP) levels ($P < .05$). Four of 32 loci (*IL12A, IL12RB1, STAT4*, and *IRF2*) associated with $ppFEV_1$ ($P < 10^{-4}$) belong to the T_H1 or IL-12 cytokine family pathway. By using a linear additive model, these 4 T_H1 pathway SNPs cumulatively explained 2.9% to 7.8% of the variance in $ppFEV_1$ values in 4 populations ($P = 3 \times 10^{-11}$). Genetic scores of these 4 SNPs were associated with $ppFEV_1$ values ($P = 2 \times 10^{-7}$) and the American Thoracic Society severe asthma classification ($P = .005$) in the Severe Asthma Research Program population. T_H2 pathway genes (*IL13, TSLP, IL33*, and *IL1RL1*) conferring asthma susceptibility were not associated with lung function.

Conclusion—Genes involved in airway structure/remodeling are associated with lung function in both general populations and asthmatic subjects. T_H1 pathway genes involved in anti-virus/bacterial infection and inflammation modify lung function in asthmatic subjects. Genes associated with lung function that might affect asthma severity are distinct from those genes associated with asthma susceptibility.

Keywords

Lung function; *FEV_1*; *asthma*; *T_H1*; IL12A; IL12RB1; STAT4; IRF2

Asthma is a chronic inflammatory respiratory disease with a high level of genetic and phenotypic heterogeneity. Phenotypically, asthma can be classified as mild, moderate, or severe according to National Asthma Education and Prevention Program, Global Initiative for Asthma, or American Thoracic Society (ATS) guidelines.[1-3] Recent work has described 5 distinct asthma severity phenotypes through cluster analysis.[4] Measures of lung function,

particularly percent predicted FEV_1 ($ppFEV_1$) values, are essential for categorizing asthma severity by using either current guidelines or cluster methodologies.[1-4]

Three recent large meta-analyses of genome-wide association studies (GWASs) in general populations of European descent have identified 28 loci for lung function[5-7]: HHIP, HTR4, INTS12-GSTCD-NPNT, TNS1, C10orf11, CDC123, MECOM, and *ZKSCAN3-ZNF323* for FEV_1 and ADAM19, DAAM2, FAM13A, GPR126, HHIP, HTR4, NOTCH4-AGER-PPT2, PID1, PTCH1, THSD4, ARMC2, CCDC38, CDC123, CFDP1, HDAC4, KCNE2, LRP1, MFAP2, MMP15, NCR3, RARB, SPATA9, and *TGFB2* for FEV_1/forced vital capacity (FVC) ratio. In a previous study of candidate genes, we have shown that *HHIP* is associated with $ppFEV_1$ and percent predicted forced vital capacity (ppFVC) values in asthmatic subjects.[8] A recent GWAS of lung function decrease has suggested that genetic profiles for longitudinal and cross-sectional lung function differ between subjects with and without asthma.[9]

In this study we performed meta-analyses of GWASs of lung function ($ppFEV_1$, ppFVC, and FEV_1/FVC ratio) in 4 European American asthmatic populations to determine whether the 28 loci identified in the previous GWASs for normal variation of lung function are important components affecting abnormal lung function in asthmatic subjects. More importantly, we explored whether some novel genes have large effects on lung function unique to asthmatic subjects.

METHODS

Study subjects

Subjects with mild-to-severe asthma recruited at the National Heart, Lung, and Blood Institute (NHLBI)–funded Severe Asthma Research Program (SARP) centers were carefully characterized, including baseline spirometry with a medication withhold before testing.[3,4,8] Similar baseline spirometry was performed in subjects with severe or difficult-to-treat asthma from The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) multicenter study.[8,10,11] Asthmatic subjects who were studied in the NHLBI's Collaborative Studies on the Genetics of Asthma (CSGA) by the Wake Forest investigators using a similar protocol were included in the analyses.[8,12]Asthmatic patients from 2 NHLBI-funded Asthma Clinical Research Network (ACRN) clinical trials (the Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroids [TALC] and Best Adjustment Strategy for Asthma in Long Term [BASALT] trials) were also included.[13] The patients from the BASALT trials (ClinicalTrials.gov no. NCT00495157) had mild-to-moderate asthma. The patients from the TALC trials (ClinicalTrials.gov no. NCT00565266) had poorly controlled disease on a lower dose of inhaled corticosteroid.[13] All studies were approved by the appropriate institutional review boards at the participating sites, including obtaining appropriate informed consent.

DNA was isolated by using standard protocols, and single nucleotide polymorphism (SNP) genotyping was performed with the Illumina HumanCNV370 BeadChip (Illumina, San Diego, Calif) for TENOR samples. SARP and CSGA samples were genotyped with the Illumina HumanHap1M BeadChip. ACRN samples were genotyped with the Illumina HumanOmniExpress700k BeadChip. Genotyping for all studies was performed with BeadStudio or GenomeStudio (Illumina).

Statistical analysis

Quality control processes of GWASs for the SARP, CSGA, and TENOR populations have been described previously.[8] Quality control of ACRN cases was performed in a similar manner.

$ppFEV_1$ and ppFVC values were calculated based on Hankinson formula.[14] Outliers (outside of 3× SDs of $ppFEV_1$, ppFVC, and FEV_1/FVC ratio values) were removed. $ppFEV_1$, ppFVC, and FEV_1/FVC ratio values were standardized with a mean of 0 and an SD of 1. A linear additive model was used for analysis of standardized $ppFEV_1$, ppFVC, and FEV_1/FVC ratio values in the SARP, CSGA, TENOR, and ACRN populations separately by using PLINK (version 1.06, http://pngu.mgh.harvard.edu/purcell/plink/)[15] adjusted for significant principal components (EIGENSTRAT, version 3.0, http://genepath.med.harvard.edu/~reich/Software.htm).[16] Association plots were generated with SNAP (version 2.0; http://www.broad.mit.edu/mpg/snap/).[17]

Meta-analyses of linear regression slope β with 369,771 SNPs that were shared in all studies from 4 European American populations (SARP, CSGA, TENOR, and ACRN) were performed, with weights proportional to the inverse variance of β using METAL software (http://www.sph.umich.edu/csg/abecasis/metal/). *P* values with genomic control adjustment from each population were used for meta-analysis to reduce genomic inflation.

Joint analysis of the 4 T_H1 pathway SNPs (rs7636840 in *IL12A*, rs388159 in *IL12RB1*, rs925847 in *STAT4*, and rs3756089 in *IRF2*) was performed in the SARP population. Genetic scores were defined by the number of risk alleles present in these 4 SNPs. A linear or logistic model was used for analysis of $ppFEV_1$ values and percentages of subjects with severe asthma by using the ATS asthma severity classification,[3] with genetic scores as defined.

RESULTS

Subjects' demographics

Although the 4 studies were all composed of European American subjects with asthma and use similar clinical approaches to phenotyping cases, they differed in the proportion of subjects with severe asthma. SARP is a cohort enriched for subjects with severe asthma but also well balanced with subjects with mild-to-moderate asthma. TENOR focused on subjects with difficult-to-treat or severe asthma. CSGA included all levels of severity but primarily milder levels. The ACRN study is composed of the TALC and BASALT trials, in which BASALT had subjects with mild-to-moderate asthma but TALC focused on subjects with poorly controlled asthma receiving a low dose of inhaled corticosteroids. These phenotypic differences are reflected in baseline lung function (see Table E1 in this article's Online Repository at www.jacionline.org). Only subjects with an age of enrollment of 12 years or greater were included in the analysis to reduce the age effect on lung development. The SARP cohort is well phenotyped with a relatively large sample size (n=618) and has a broad range of lung function, and thus SARP was used as the primary population for this analysis.

After a quality control process, 1,544 subjects from the 4 studies were analyzed with the shared 369,771 SNPs for association with predrug spirometric measures of lung function: $ppFEV_1$, ppFVC, and FEV_1/FVC ratio. $ppFEV_1$ was the primary phenotype in this study because it is the lung function measure most used for clinical studies in asthmatic subjects.

Meta-analysis of GWASs of ppFEV$_1$

Forty-four SNPs from 32 independent loci were suggestively associated with ppFEV$_1$ values ($P < 10^{-4}$; Fig 1 and see Table E2 and Fig E1 in this article's Online Repository at www.jacionline.org). rs7670758, which is located approximately 60 kb upstream of *HHIP*, was associated with ppFEV$_1$ values ($P = 9.5 \times 10^{-5}$). The minor allele A of rs7670758 was associated with decreased ppFEV$_1$ values. This effect direction for rs7670758 is consistent with previous findings in general populations,[5-7] supporting our study design and statistical analysis.

Four of 32 loci associated with ppFEV$_1$ values were within or near important T$_H$1 or IL-12 cytokine family pathway genes: *IL12A* (rs7636840 and rs11918254 in strong linkage disequilibrium [LD]: $r^2 = 0.94$), *IL12RB1* (rs388159), *STAT4* (rs925847), and *IRF2* (rs3756089; Table I and see Table E2 and Figs E2-E5 in this article's Online Repository at www.jacionline.org). Four T$_H$1 pathway SNPs were consistently associated with ppFEV$_1$ values in the SARP, CSGA, TENOR, and ACRN populations (Tables I and II and see Table E3 in this article's Online Repository at www.jacionline.org).

These 4 T$_H$1 pathway SNPs cumulatively explained 2.9% to 7.8% of the variance in ppFEV$_1$ values in 4 populations, with a meta-analysis *P* value of 3×10^{-11} (Table II). The possession of an increased number of risk alleles (genetic scores) for these 4 SNPs inversely correlated with ppFEV$_1$ values ($P = 2 \times 10^{-7}$) and positively correlated with the ATS asthma severity classification ($P = .005$) in the SARP population (Fig 2). In the SARP cohort ppFEV$_1$ values displayed a stepwise decrease from 81.9 (SD, 20.1) to 76.6 (SD, 21.1) and then to 65.1 (SD, 22.3), and the percentage of subjects with severe asthma (based on ATS classification) showed an increase from 40.2% to 49.4% and then to 64.3% with the increasing number of risk alleles (Fig 2).

Meta-analyses of GWASs of ppFVC and FEV$_1$/FVC ratio values

Thirty-nine SNPs were suggestively associated with ppFVC values ($P < 10^{-4}$, see Table E4 in this article's Online Repository at www.jacionline.org). Two SNPs downstream of *IL12RB1* (rs12984174 and rs388159 in moderate LD: $r^2 = 0.75$) ranked as number 1 and number 9. rs388159 was also the top candidate SNP associated with ppFEV$_1$ values (Table I), making it the most robust finding in this study.

Thirty-one SNPs were suggestively associated with FEV$_1$/FVC ratio values ($P < 10^{-4}$, see Table E5 in this article's Online Repository at www.jacionline.org). rs11032873, which is located between *APIP* and *EHF*, was associated with FEV$_1$/FVC ratio values ($P = 3.1 \times 10^{-5}$). The *APIP-EHF* region has been identified as a modifier locus of lung disease severity in patients with cystic fibrosis.[18] Two adenylate cyclase family genes (*ADCY2* and *ADCY9*) were associated with FEV$_1$/FVC ratio values ($P = 5.6 \times 10^{-5}$ or 6.3×10^{-5} for rs12659620 or rs2230739, respectively). rs3130696, which is located upstream of *HLA-C*, was associated with FEV$_1$/FVC ratio values ($P = 7.3 \times 10^{-5}$).

Replication of 28 normal lung function loci

Two meta-analyses of GWASs in approximately 20,000 healthy subjects of European descent have identified 12 loci for lung function[6,7]: *HHIP, HTR4, INTS12-GSTCD-NPNT*, and *TNS1* for FEV$_1$ and ADAM19, DAAM2, FAM13A, GPR126, HHIP, HTR4, NOTCH4-AGER-PPT2, PID1, PTCH1, and *THSD4* for FEV$_1$/FVC ratio (see Table E6 in this article's Online Repository at www.jacionline.org). One more recent meta-analysis of GWASs in approximately 90,000 healthy subjects of European descent has identified 16 novel loci for lung function[5]: *C10orf11, CDC123, MECOM*, and *ZKSCAN3-ZNF323* for FEV$_1$ and ARMC2, CCDC38, CDC123, CFDP1, HDAC4, KCNE2, LRP1, MFAP2, MMP15, NCR3,

RARB, SPATA9, and *TGFB2* for FEV_1/FVC ratio (see Table E7 in this article's Online Repository at www.jacionline.org).

In this study we replicated 7 of 28 lung function loci at SNP levels ($P < .05$) for the same phenotypes and in the same effect direction in asthma: *HHIP* (rs1980057 and rs7670758), *FAM13A* (rs2869967 and rs6825998), *THSD4* (rs12899618), *GSTCD* (rs17035917 and rs6820671), *NOTCH4-AGER* (rs206015), *RARB* (rs1529672 and rs7616278), and *ZNF323* (rs6922111 and rs1416920, see Tables E6 and E7).

Comparison of asthma genes and lung function genes

Although lung function is an essential intermediate phenotype distinct between asthmatic patients and healthy subjects, the genes associated with lung function were largely not associated with asthma susceptibility in the GABRIEL study (extracted from the European Genome-Phenome Archive; http://www.cng.fr/gabriel; accession no. EGAS00000000077).[19]

In this study the 4 T_H1 pathway SNPs associated with lung function were not associated with asthma susceptibility in the GABRIEL,[19] TENOR,[11] or SARP/CSGA[20] studies (see Table E8 in this article's Online Repository at www.jacionline.org). Six SNPs (in *ORMDL3-GSDMB, IL33, IL1RL1-IL18R1, TSLP, IL13*, and *HLA-DRA*) associated with asthma susceptibility[19-21] were not associated with $ppFEV_1$ values (see Table E9 in this article's Online Repository at www.jacionline.org).

Two-step asthma progression genetic model

On the basis of this study and previous findings, we suggest a 2-step asthma progression genetic model (Fig 3). In the first step genetic variants in T_H2 pathway genes (*IL13, TSLP, IL33*, and *IL1RL1*), interacting with environmental factors, induce T_H2-dominant response and atopy, which distinguish subjects with asthma from healthy subjects. In the second step genetic variants in the T_H1 or IL-12 cytokine family pathway (*IL12A, IL12RB1, STAT4*, and *IRF2*), airway structure/remodeling (*HHIP, FAM13A, THSD4, GSTCD, NOTCH4-AGER, RARB*, and *ZNF323*), and/or other mechanisms interacting with environmental factors lead to lower lung function and increased asthma severity.

DISCUSSION

In this study we investigated genetic variants related to lung function in 4 European American asthmatic populations (n = 1544) using GWASs and meta-analyses. Although the sample size is relatively small compared with that of the studies in general populations, the current study is the first and largest GWAS of lung function in comprehensively phenotyped subjects with asthma. Further replication in well-phenotyped independent populations is essential to confirm our findings. Three predrug spirometric measures of lung function ($ppFEV_1$, ppFVC, and FEV_1/FVC ratio) were studied. FEV_1 is the volume of air that can be expired forcibly in 1 second after full inspiration. FVC is the volume of air that can be expired forcibly after full inspiration. The FEV_1/FVC ratio is the ratio of FEV_1 to FVC. Both FEV_1 and FVC values can be decreased in patients with respiratory diseases but might not be in the same proportion. These 3 measures are correlated but might reflect fine differences in lung function. The FEV_1/FVC ratio and FEV_1 reflect airway obstruction and the grade of airway obstruction and thus are used together to define chronic obstructive pulmonary disease. $ppFEV_1$ was the primary phenotype in this study because it is highly correlated with asthma severity and mostly used for clinical studies in asthmatic subjects. Although no SNP reached genome-wide significance ($P = 5 \times 10^{-8}$), multiple SNPs were suggestively associated with $ppFEV_1$, ppFVC, or FEV_1/FVC ratio values ($P < 10^{-4}$, see

Tables E2, E4, and E5). In general, the results for these 3 measurements agreed well, although the top SNPs were not always the same in the 3 measurements (see Table E2). More importantly, multiple top SNPs belong to the same pathway and are functionally related to lung function. The same effect direction of these SNPs in all 4 studied populations further supports our results. Although a single SNP with a modest *P* value does not meet the strict requirements for association in a GWAS analysis, multiple independent loci mapped to the same biological pathway are very unlikely to be a result of chance, and thus pathway analysis is an important complementary approach to a GWAS.[22]

For example, 4 of 32 loci associated with $ppFEV_1$ values are T_H1 or IL-12 cytokine family pathway genes: *IL12A, IL12RB1, STAT4*, and *IRF2* (Table I). Although these 4 SNPs were not significant individually at the genome-wide level, cumulatively they explained 2.9% to 7.8% of the variance in $ppFEV_1$ values ($P = 3 \times 10^{-11}$, Table II). In the well-phenotyped SARP population genetic scores of these 4 SNPs were inversely associated with $ppFEV_1$ values ($P = 2 \times 10^{-7}$) and positively associated with the ATS asthma severity classification ($P = .005$). The risk allele of *IL12RB1, STAT4*, and *IRF2* is its major allele, and the risk allele of *IL12A* is its minor allele. In general, the combinations of risk alleles involving *IRF2* were correlated with higher $ppFEV_1$ values, and those including *IL12A* were associated with lower $ppFEV_1$ values (see Table E10 in this article's Online Repository at www.jacionline.org). IL-12 is a key cytokine that modulates innate and adaptive immune responses. IL-12 is a heterodimer composed of the p35 subunit (encoded by *IL12A*) and the p40 subunit (encoded by *IL12B*). The coexpression and dimerization of the IL-12RB1 and IL-12RB2 proteins led to the formation of the high-affinity IL-12 receptor. The main role of IL-12 is to activate IFN-γ (IFNG) production. The response of lymphocytes to IL-12 is mediated by STAT4, which induces the expression of IL-12RB2 and IRF1. IRF1 is a transcription activator of the genes induced by IFN-α (IFNA1), IFN-β (IFNB1), and IFNG, whereas IRF2 competitively inhibits the expression of genes activated by IRF1. The IL-12–STAT4–IFNG signaling pathway is essential for the differentiation of naive T_H cells into T_H1 cells. GWASs in patients with autoimmune diseases have consistently identified IL-12–STAT4–IFNG signaling pathway genes. For example, SNPs in the *IL12A* region have been associated with celiac disease,[23,24] primary biliary cirrhosis,[25-27] and multiple sclerosis.[28] SNPs in the *STAT4* region have been associated with systemic lupus erythematosus,[29-31] systemic sclerosis,[32] rheumatoid arthritis,[33,34] and primary biliary cirrhosis.[27] Candidate gene studies have indicated that *IL12RB1, STAT4*, and *IRF2* were associated with asthma, but the association results are not consistent.[35]

Several genes associated with lung development or height were identified (see Tables E2, E4, and E5), including *HHIP*,[5-7] *SULF1*,[36] and *SYN3*,[36,37] although $ppFEV_1$ and ppFVC values were already adjusted for height. Similarly, GWASs of lung function in general populations also identified multiple genes associated with height,[5-7] indicating the importance of height in determining lung volume or pulmonary function.

Two adenylate cyclase family genes (*ADCY2* and *ADCY9*) were suggestively associated with FEV_1/FVC ratio values (see Table E5). The same nonsynonymous coding SNP (rs2230739 or Ile772Met) in *ADCY9* has been shown to be significantly associated with the difference in improvement of FEV_1 values with the response to β-agonist with or without corticosteroid treatment.[38] In a candidate gene study *ADCY2* was associated with both chronic obstructive pulmonary disease and FEV_1/FVC ratio values independently of smoking effect.[39]

Previously, we have shown that a subset of genes that regulate lung function in general populations might be associated with abnormal lung function in subjects with asthma.[8] In this study this list has been extended to 7 of 28 lung function loci at the SNP level: *HHIP*

(rs1980057 and rs7670758), *FAM13A* (rs2869967 and rs6825998), *THSD4* (rs12899618), *GSTCD* (rs17035917 and rs6820671), *NOTCH4-AGER* (rs206015), *RARB* (rs1529672 and rs7616278), and *ZNF323* (rs6922111 and rs1416920, see Tables E6 and E7). Among these 7 genes, several might be involved in airway structure and airway remodeling: (1) *HHIP* might influence embryonic lung-branching morphogenesis[40]; (2) *RARB* is an inhibitor of the perinatal formation of pulmonary alveoli[41]; and (3) *NOTCH4* and *THSD4* might be involved in angiogenesis and airway remodeling.[42,43] Furthermore, both *TIMP3* and *CITED2*, which are associated with ppFEV1 values (see Table E2), might be involved in hypoxia-inducible factor 1α/vascular endothelial growth factor–induced hypoxia and angiogenesis.[44,45]

In the GABRIEL study[19] the 12 lung function loci[6,7] were not associated with asthma susceptibility. In this study the 4 T_H1 pathway SNPs associated with lung function were not associated with asthma susceptibility (see Table E8); conversely, 6 SNPs (in *ORMDL3-GSDMB, IL33, IL1RL1-IL18R1, TSLP, IL13*, and *HLA-DRA*) conferring asthma susceptibility[19-21] were not associated with $ppFEV_1$ values (see Table E9). These results indicate that genes associated with lung function that can modify asthma severity might be distinct from those genes associated with asthma susceptibility. Thus we hypothesize that asthma would develop in a subject with susceptibility loci but that more severe asthma with reduced lung function requires the presence of additional severity or pulmonary function loci.

GWASs in subjects with autoimmune diseases have consistently identified *IL12A*[23-28] and *STAT4*[27,29-34] regions. The top SNPs of *STAT4* identified in autoimmune diseases are not in strong LD with the top SNPs identified in this study, indicating that distinct transcription regulation mechanisms might exist in different tissues for autoimmune diseases and asthma. However, evidence from genetic association studies indicates the opposite direction of effect size for *STAT4* between autoimmune diseases and $ppFEV_1$ values. For example, the minor allele T of rs10168266 in *STAT4* is the risk allele for primary biliary cirrhosis ($P = 4.2 \times 10^{-5}$)[24] but the protective allele for $ppFEV_1$ ($P = .028$). The minor allele A of rs3821236 is the risk allele for systemic lupus erythematosus ($P = 8.5 \times 10^{-11}$)[30] but the protective allele for $ppFEV_1$ ($P = .036$). The minor allele T of rs7574865 is the risk allele for systemic lupus erythematosus in subjects of Chinese ($P = 5.2 \times 10^{-42}$)[31] or European ($P = 9.0 \times 10^{-14}$)[29] descent but possibly the protective allele for $ppFEV_1$ ($P = .093$). The minor allele T of rs925847 is the protective allele for $ppFEV_1$ ($P = 8.2 \times 10^{-5}$) and eczema ($P = .014$)[46] but the major risk allele for ulcerative colitis in the Korean population ($P = .025$).[47] In general, the expression of T_H1 pathway genes is believed to be increased in subjects with autoimmune diseases, and thus the expression of T_H1 pathway genes might be positively correlated with $ppFEV_1$ values or negatively correlated with asthma severity (Fig 3).

The T_H1 pathway primarily acts against viruses and bacterial infections. Respiratory tract virus infection might initiate and exacerbate asthma. The decrease in production of IFN-γ in response to viral infection of monocytes at birth predicts the susceptibility to respiratory tract illness during the first year of life.[48] The weaker T_H1 responses to viral infection are associated with asthma exacerbations and suggest that viral infection can exacerbate a pre-existing T_H2-dominated lung disease.[49] In a study of acute asthma exacerbations in children,[50] the expression of T_H1 pathways genes is decreased during exacerbation, with deficits in baseline lung function. Rhinovirus-induced clinical phenotypes are more severe in asthmatic subjects than healthy control subjects and indicate impaired T_H1 or augmented T_H2 immune responses.[51] The weaker T_H1 response might strengthen the T_H2-dominated response in asthmatic subjects, whereas the impaired T_H1 response against viral infection might extend the inflammation process and lead to airway remodeling (Fig 3).

We must emphasize the complexity of immunologic pathways. Although we speculated that the T_H1 pathway might be important for lung function in asthmatic subjects, the genes identified (*IL12A, IL12RB1, STAT4*, and *IRF2*) could be involved in other pathways. For example, IL-12p35, encoded by *IL12A*, is a ligand subunit shared by IL-12 and IL-35, where IL-35 might be involved in the regulatory T-cell pathway.[52] IL-12Rβ1, which is encoded by *IL12RB1*, is a receptor subunit shared by IL-12 and IL-23, where IL-23 is a proinflammatory cytokine involved in the T_H17 pathway.[52] More comprehensively, we can label *IL12A, IL12RB1, STAT4*, and *IRF2* as IL-12 cytokine family pathway genes.

On the basis of this study and previous findings, we propose a simplified 2-step genetic model of asthma progression (Fig 3). Genetic variants in T_H2 pathway genes (*IL13, TSLP, IL33*, and *IL1RL1*) induce T_H2-dominant response, atopy, and asthma susceptibility. Genetic variants in T_H1 or IL-12 cytokine family pathway genes (*IL12A, IL12RB1, STAT4*, and *IRF2*), airway structure/remodeling (*HHIP, FAM13A, THSD4, GSTCD, NOTCH4-AGER, RARB*, and *ZNF323*), and/or other mechanisms affect lung function and increase asthma severity and asthma exacerbation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ACRN	Asthma Clinical Research Network
ATS	American Thoracic Society
BASALT	Best Adjustment Strategy for Asthma in Long Term
CSGA	Collaborative Studies on the Genetics of Asthma
FVC	Forced vital capacity
GWAS	Genome-wide association study
LD	Linkage disequilibrium
NHLBI	National Heart, Lung, and Blood Institute
ppFEV$_1$	Percent predicted FEV$_1$
ppFVC	Percent predicted forced vital capacity
SARP	Severe Asthma Research Program
SNP	Single nucleotide polymorphism

TALC	Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroids
TENOR	The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens

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Key messages

- Genes involved in airway structure/remodeling are associated with lung function in both general populations and subjects with asthma.
- T_H1 pathway genes involved in anti-virus/bacterial infection and inflammation modify lung function in asthmatic subjects.

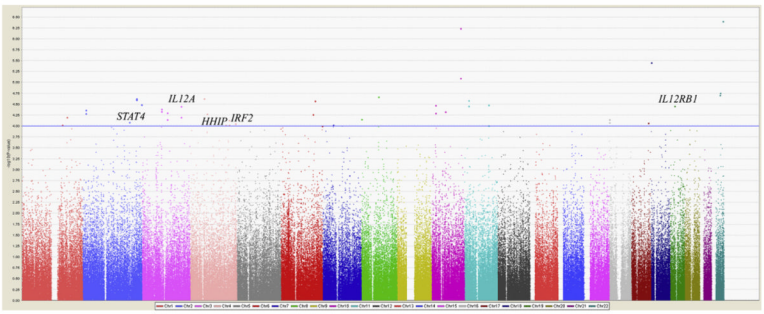

FIG 1.

Genome-wide association of ppFEV$_1$ values with 369,771 SNPs in 4 European American populations. The *color scale* of the *x-axis* represents chromosomes. Negative log-transformed meta-analysis *P* values are shown on the *y-axis*. The *blue horizontal line* is drawn at a *P* value of 10^{-4}.

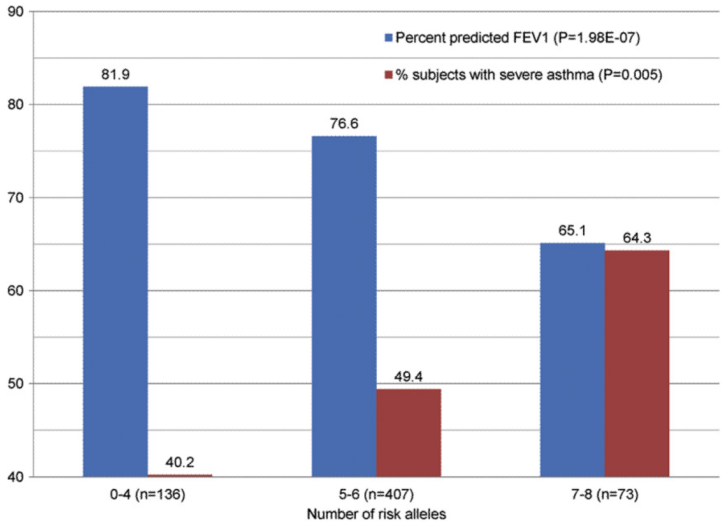

FIG 2.

Joint analysis of 4 T_H1 pathway SNPs in *IL12A, IL12RB1, STAT4*, and *IRF2* for $ppFEV_1$ values in the SARP population. *Blue bars* represent $ppFEV_1$, and *purple bars* represent percentages of subjects with severe asthma based on ATS classification.

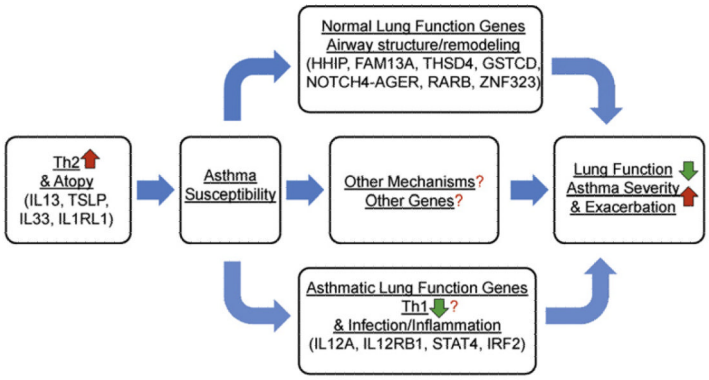

FIG 3.

Two-step asthma progression genetic model. The *red arrow* indicates higher gene expression levels or protein activities. The *green arrow* indicates lower gene expression levels or protein activities. The *question mark* indicates the lack of available experimental evidence.

TABLE I

Meta-analysis results of 4 T_H1 pathway SNPs associated with $ppFEV_1$

SNP	Gene	Chromosome	BP	Location	Ref/alt allele	Alt freq	Alt effect	Direction*	*P* value
rs388159	*IL12RB1*	19	18002996	3′†	C/T	0.1767	0.1943	++++	3.47E-05
rs7636840	*IL12A*	3	161161335	5′‡	G/T	0.1725	−0.1945	− − − −	3.58E-05
rs925847	*STAT4*	2	191605785	Intron	C/T	0.2774	0.1579	++++	8.17E-05
rs3756089	*IRF2*	4	185552786	Intron	C/T	0.0818	0.2595	++++	8.79E-05

*Direction: +/− indicates that the alternative allele is correlated with $ppFEV_1$ values positively/negatively in the 4 cohorts, and the order of the populations is SARP, CSGA, ACRN, and TENOR.

†rs388159 is located between *IL12RB1* and *ARDDC2*.

‡rs7636840 is located between *IL12A* and *SCHIP1*.

TABLE II

Four T_H1 pathway SNPs in a prediction model for $ppFEV_1$ values

SNP	Gene	SARP	CSGA	ACRN	TENOR
rs7636840	*IL12A*	0.0031	0.93	0.0031	0.15
rs388159	*IL12RB1*	0.0028	0.28	0.15	0.011
rs925847	*STAT4*	0.032	0.00087	0.49	0.055
rs3756089	*IRF2*	0.009	0.079	0.0035	0.45
Linear regression (*P* value/R^2) *		9.30E-06/.046	0.0043/.078	0.00063/.065	0.013/.029
Meta-analysis *P* value †			3.31E-11		

* Linear regression (*P* value/R^2): linear regression of $ppFEV_1$ values versus 4 T_H1 pathway SNPs.

† Meta-analysis *P* value: Meta-analysis of *P* values with weights proportional to sample size.