

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

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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 FEV₁ [ppFEV₁], percent predicted forced

vital capacity, and FEV₁/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

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ppFEV₁ ($P < 10^{-4}$) 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 ppFEV₁ values in 4 populations ($P = 3 \times 10^{-11}$). Genetic scores of these 4 SNPs were associated with ppFEV₁ 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 *ILIRLI*) 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. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: Lung function, FEV₁, 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 FEV₁ (ppFEV₁) 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 function⁵⁻⁷: *HHIP*, *HTR4*, *INTS12-GSTCD-NPNT*, *TNS1*, *C10orf11*, *CDC123*, *MECOM*, and *ZKSCAN3-ZNF323* for FEV₁ 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 FEV₁/forced vital capacity (FVC) ratio. In a previous study of candidate genes, we have shown that *HHIP* is associated with ppFEV₁ 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 (ppFEV₁, ppFVC, and FEV₁/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

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

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 HumanHapIM 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.

ppFEV₁ and ppFVC values were calculated based on Hankinson formula.¹⁴ Outliers (outside of 3× SDs of ppFEV₁, ppFVC, and FEV₁/FVC ratio values) were removed. ppFEV₁, ppFVC, and FEV₁/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 ppFEV₁, ppFVC, and FEV₁/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://genenpath.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 ppFEV₁ 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: ppFEV₁, ppFVC, and FEV₁/FVC ratio. ppFEV₁ 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 ppFEV₁

Forty-four SNPs from 32 independent loci were suggestively associated with ppFEV₁ 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 ppFEV₁ values ($P = 9.5 \times 10^{-5}$). The minor allele A of rs7670758 was associated with decreased ppFEV₁ 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 ppFEV₁ values were within or near important T_H1 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_H1 pathway SNPs were consistently associated with ppFEV₁ 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_H1 pathway SNPs cumulatively explained 2.9% to 7.8% of the variance in ppFEV₁ 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 ppFEV₁ 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 ppFEV₁ 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 FEV₁/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 ppFEV₁ values (Table I), making it the most robust finding in this study.

Thirty-one SNPs were suggestively associated with FEV₁/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 FEV₁/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 FEV₁/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 FEV₁/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 *TNSI* for FEV₁ and *ADAM19*, *DAAM2*, *FAM13A*, *GPR126*, *HHIP*, *HTR4*, *NOTCH4-AGER-PPT2*, *PID1*, *PTCH1*, and *THSD4* for FEV₁/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 function⁵: *C10orf11*, *CDC123*, *MECOM*, and *ZKSCAN3-ZNF323* for FEV₁ and *ARMC2*, *CCDC38*, *CDC123*, *CFDP1*, *HDAC4*, *KCNE2*, *LRP1*, *MFAP2*, *MMP15*, *NCR3*, *RARB*, *SPATA9*, and *TGFB2* for FEV₁/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

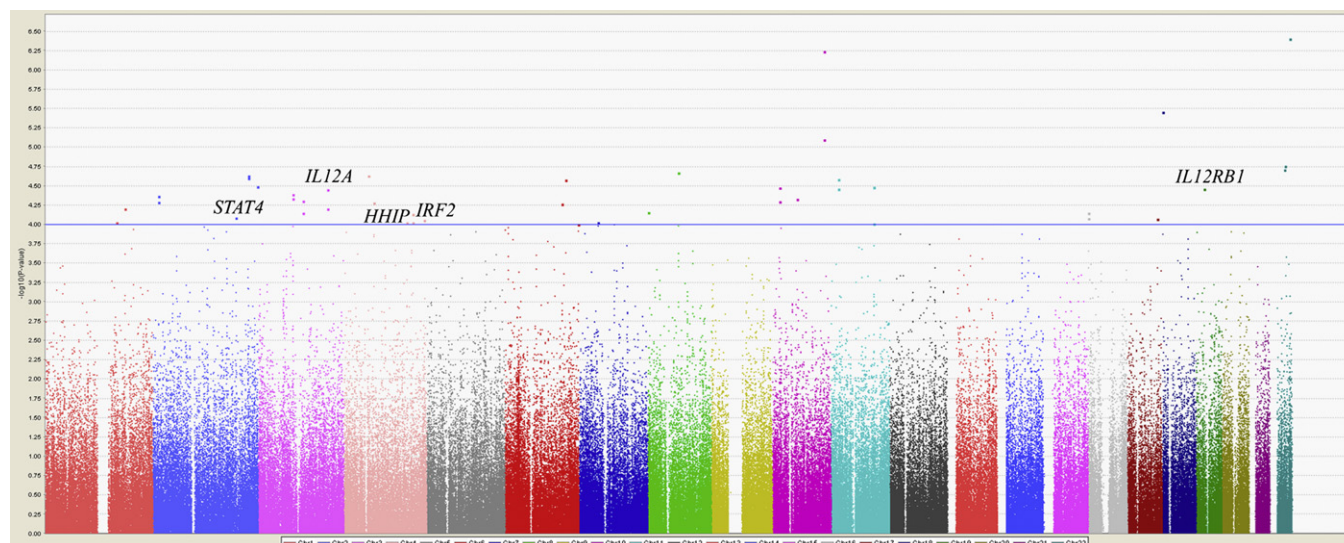


FIG 1. Genome-wide association of ppFEV₁ values with 369,771 SNPs in 4 European American populations. The color scale of the x-axis represents chromosomes. Negative log-transformed meta-analysis P values are shown on the y-axis. The blue horizontal line is drawn at a P value of 10^{-4} .

TABLE I. Meta-analysis results of 4 T_H1 pathway SNPs associated with ppFEV₁

SNP	Gene	Chromosome	BP	Location	Ref/alt allele	Alt freq	Alt effect	Direction*	P value
rs388159	<i>IL12RB1</i>	19	18002996	3'†	C/T	0.1767	0.1943	++++	3.47E-05
rs7636840	<i>IL12A</i>	3	161161335	5'‡	G/T	0.1725	-0.1945	----	3.58E-05
rs925847	<i>STAT4</i>	2	191605785	Intron	C/T	0.2774	0.1579	++++	8.17E-05
rs3756089	<i>IRF2</i>	4	185552786	Intron	C/T	0.0818	0.2595	++++	8.79E-05

*Direction: +/- indicates that the alternative allele is correlated with ppFEV₁ 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 ppFEV₁ values

SNP	Gene	SARP	CSGA	ACRN	TENOR
rs7636840	<i>IL12A</i>	0.0031	0.93	0.0031	0.15
rs388159	<i>IL12RB1</i>	0.0028	0.28	0.15	0.011
rs925847	<i>STAT4</i>	0.032	0.00087	0.49	0.055
rs3756089	<i>IRF2</i>	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/R²): linear regression of ppFEV₁ values versus 4 T_H1 pathway SNPs.

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

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/CSGA²⁰ 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 susceptibility¹⁹⁻²¹ were not associated with ppFEV₁ 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

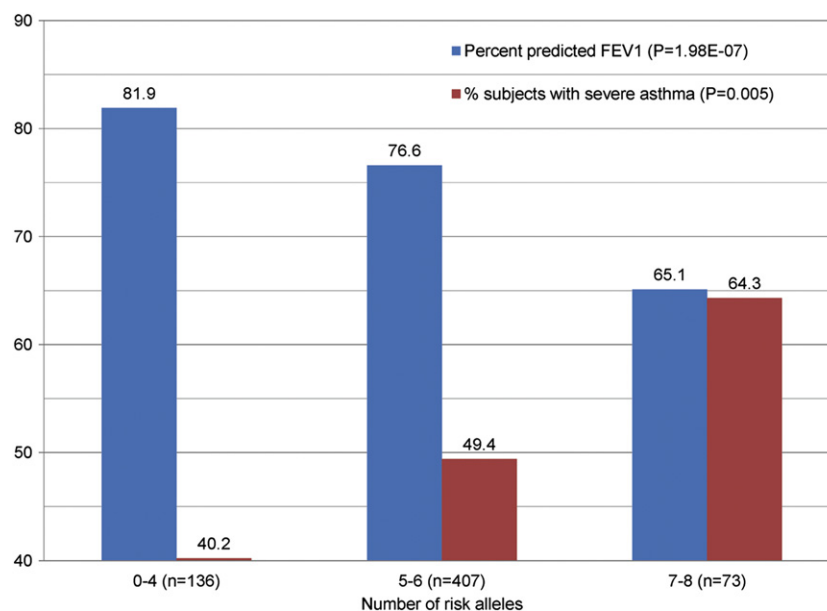


FIG 2. Joint analysis of 4 T_H1 pathway SNPs in *IL12A*, *IL12RB1*, *STAT4*, and *IRF2* for ppFEV₁ values in the SARP population. Blue bars represent ppFEV₁, and purple bars represent percentages of subjects with severe asthma based on ATS classification.

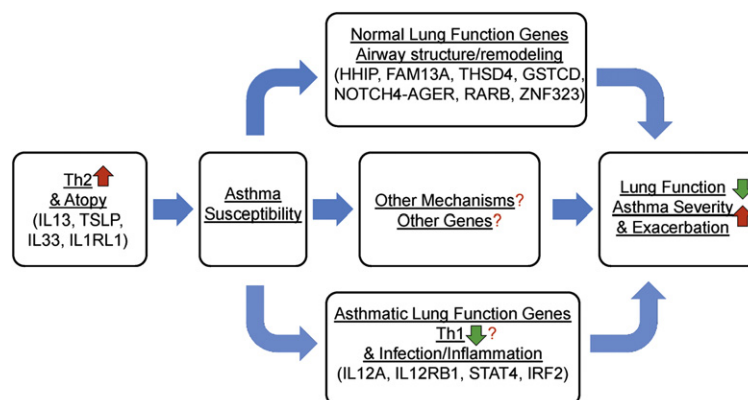


FIG 3. Two-step asthma progression genetic model. The red arrow indicates higher gene expression levels or protein activities. The green arrow indicates lower gene expression levels or protein activities. The question mark indicates the lack of available experimental evidence.

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 (ppFEV₁, ppFVC, and FEV₁/FVC ratio) were studied. FEV₁ 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 FEV₁/FVC ratio is the ratio of FEV₁ to FVC. Both FEV₁ 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 FEV₁/FVC ratio and FEV₁ reflect airway obstruction and the grade of airway obstruction and thus are used together to define chronic obstructive pulmonary disease. ppFEV₁ 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 ppFEV₁, ppFVC, or FEV₁/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 ppFEV₁ 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 ppFEV₁ values ($P = 3 \times 10^{-11}$, Table II). In the well-phenotyped SARP population genetic scores of these 4 SNPs were inversely associated with ppFEV₁ 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 ppFEV₁ values, and those including *IL12A* were associated with lower ppFEV₁ 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 ppFEV₁ 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 FEV₁/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 FEV₁ 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 FEV₁/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 morphogenesis⁴⁰;

(2) *RARB* is an inhibitor of the perinatal formation of pulmonary alveoli⁴¹; 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 1 α /vascular endothelial growth factor–induced hypoxia and angiogenesis.^{44,45}

In the GABRIEL study¹⁹ 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 ppFEV₁ 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 ppFEV₁ 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 ppFEV₁ ($P = .028$). 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 ppFEV₁ ($P = .036$). 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 ppFEV₁ ($P = .093$). The minor allele T of rs925847 is the protective allele for ppFEV₁ ($P = 8.2 \times 10^{-5}$) 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 ppFEV₁ 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 pathway 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-12R β 1, 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.

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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.

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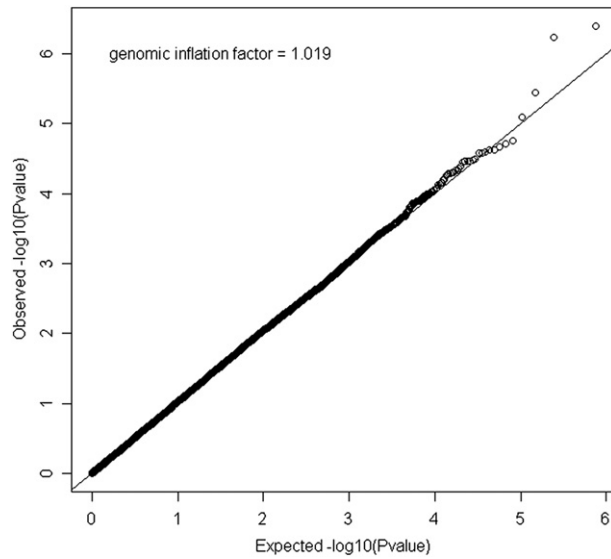


FIG E1. QQ plot of genome-wide association of ppFEV₁ values with 369,771 SNPs in 4 European American populations. Negative log-transformed expected *P* values are shown on the *x*-axis. Negative log-transformed observed *P* values are shown on the *y*-axis.

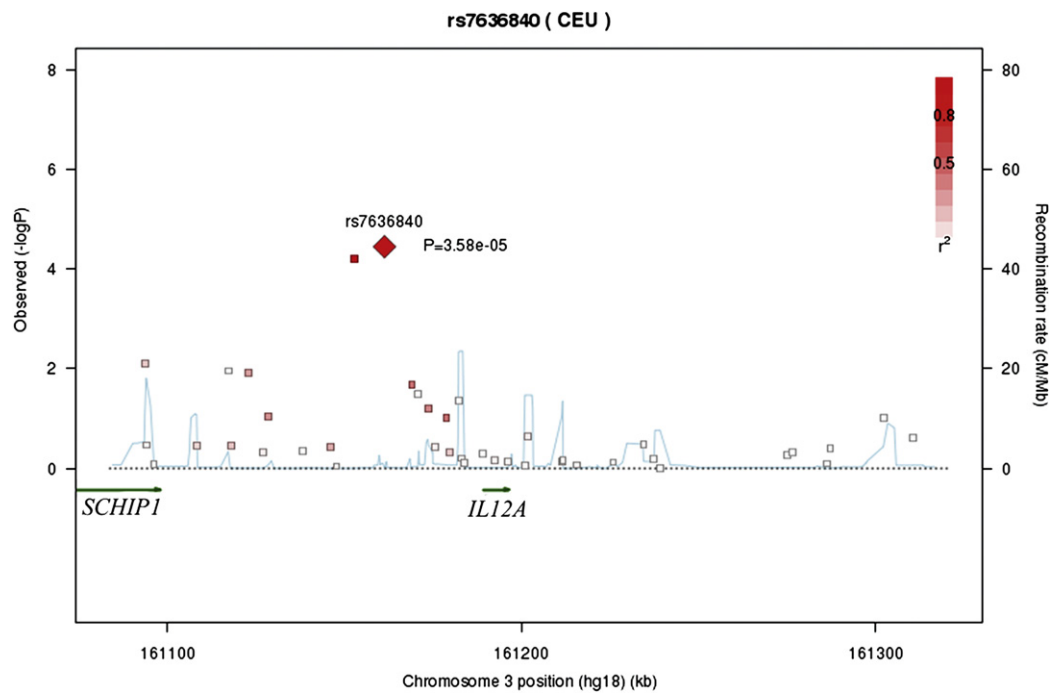


FIG E2. Association plot of the *IL12A* region. Negative log-transformed *P* value (left) and recombination rate (right) are shown. The red color scale represents the strength of LD of the SNPs with rs7636840.

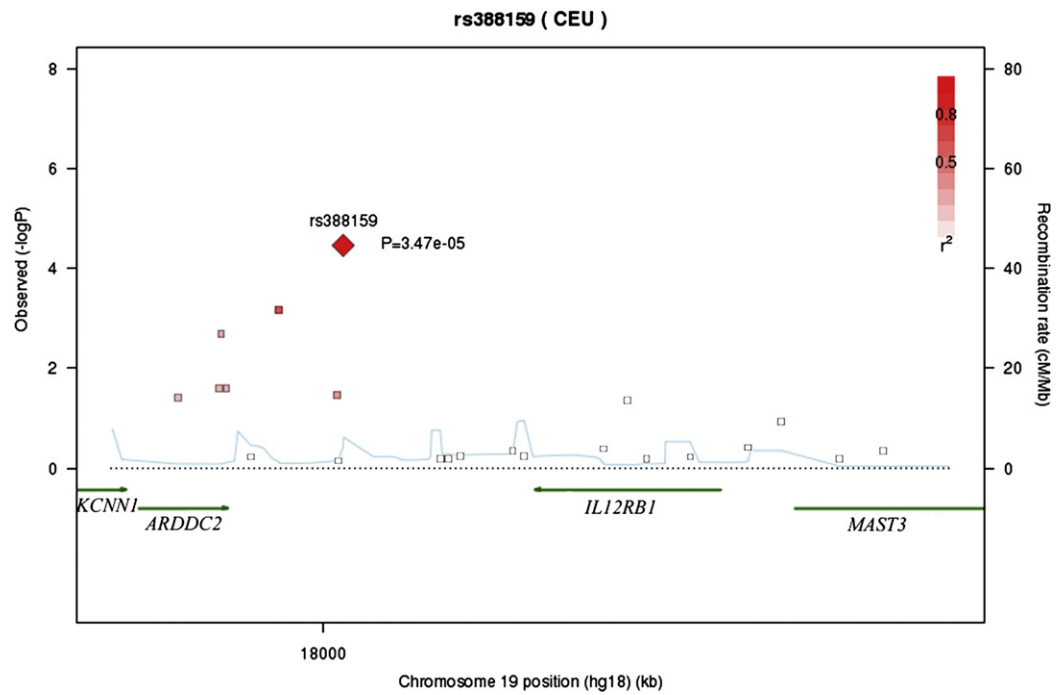


FIG E3. Association plot of the *IL12RB1* region. Negative log-transformed *P* value (left) and recombination rate (right) are shown. The red color scale represents the strength of LD of the SNPs with rs388159.

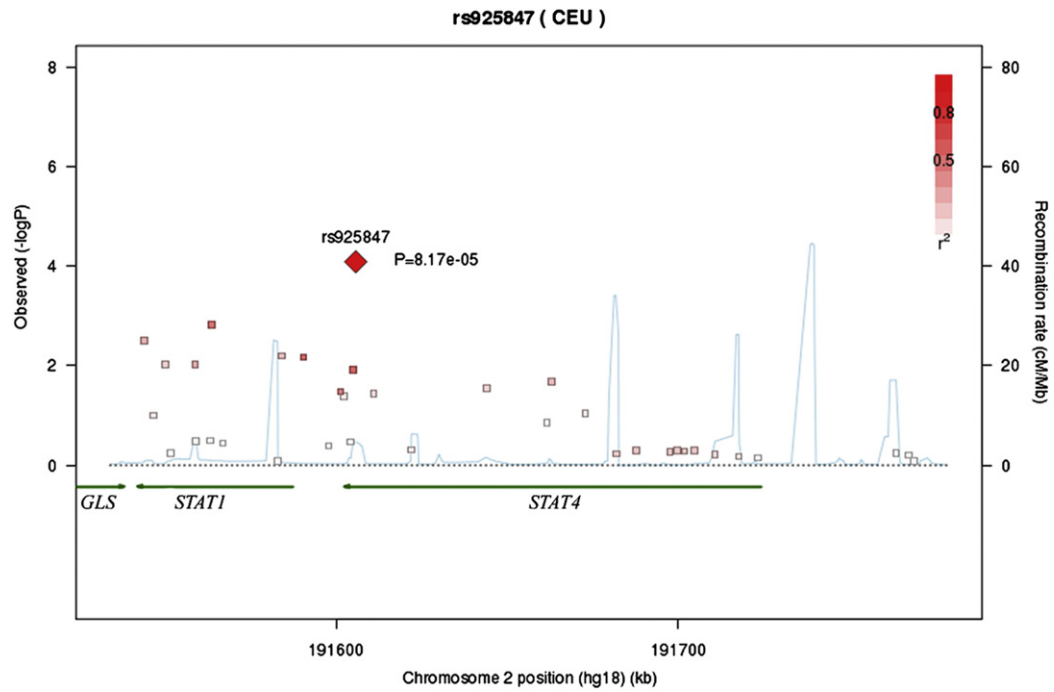


FIG E4. Association plot of the *STAT4* region. Negative log-transformed *P* value (left) and recombination rate (right) are shown. The red color scale represents the strength of LD of the SNPs with rs925847.

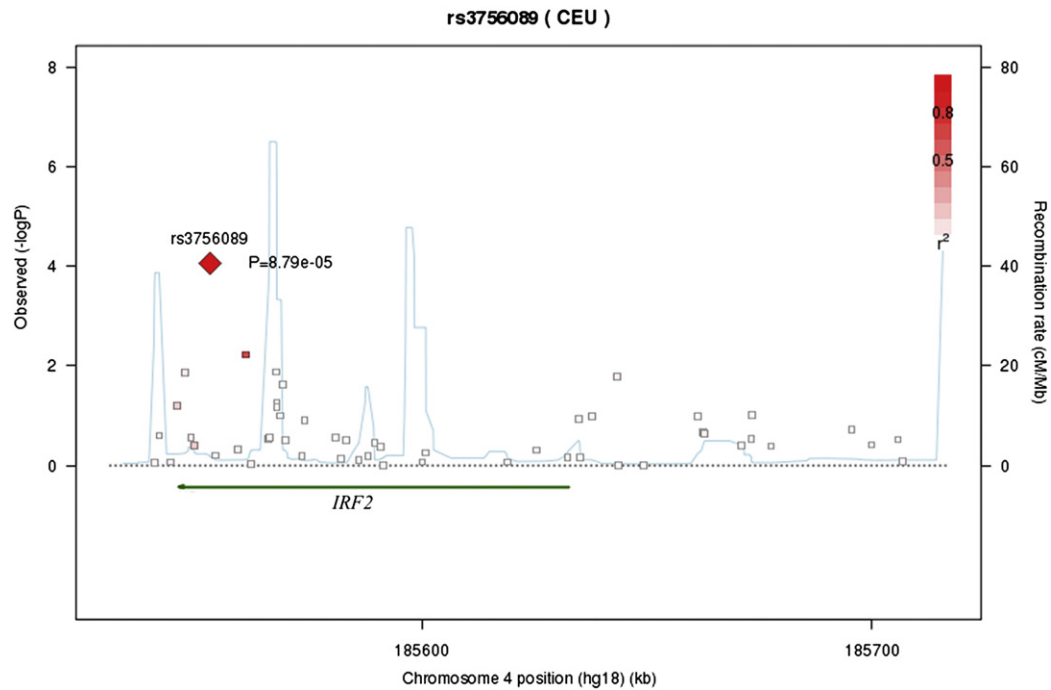


FIG E5. Association plot of the *IRF2* region. Negative log-transformed *P* value (*left*) and recombination rate (*right*) are shown. The red color scale represents the strength of LD of the SNPs with rs3756089.

TABLE E1. Demographics (means $\pm$ SDs) of subjects in SARP, CSGA, ACRN, and TENOR

	SARP	CSGA	ACRN	TENOR
No.	618	193	296	437
Age (y)	38.8 $\pm$ 14.3	29.0 $\pm$ 12.6	38.2 $\pm$ 12.7	46.8 $\pm$ 18.2
Sex (% female)	65	67	68	63
ppFEV ₁	76.3 $\pm$ 21.5	82.4 $\pm$ 17.6	82.3 $\pm$ 14.8	78.5 $\pm$ 21.1
ppFVC	86.4 $\pm$ 18.2	91.2 $\pm$ 15.5	NA	89.0 $\pm$ 17.8
FEV ₁ /FVC ratio	0.71 $\pm$ 0.12	0.76 $\pm$ 0.11	0.72 $\pm$ 0.10	0.72 $\pm$ 0.12

TABLE E2. Meta-analysis results of the top 44 SNPs ($P < 10^{-4}$) associated with ppFEV₁

SNP	Gene	Chromosome	BP	Location	Ref/alt allele	Alt freq	Alt effect	Direction	ppFEV ₁	ppFVC	FEV ₁ /FVC ratio
rs58667	UPK3A	22	44046676	5'	G/A	0.38	0.18	++++	3.95E-07	3.79E-04	6.86E-03
rs4752066	EMX2	10	119217332	5'	G/T	0.10	-0.30	-----	5.82E-07	2.79E-03	2.92E-04
rs1291183	YES1	18	839178	5'	G/T	0.30	-0.18	-----	3.54E-06	5.47E-05	1.18E-02
rs242966	EMX2	10	119224776	5'	G/A	0.88	0.25	++++	8.02E-06	7.24E-03	7.86E-04
rs5755023	LARGE	22	33129261	5'	G/A	0.83	-0.21	-----	1.75E-05	1.81E-03	2.29E-03
rs2040403	SYN3	22	31809580	5'	G/A	0.92	0.29	++++	1.96E-05	9.96E-03	1.34E-03
rs7836170	SULF1	8	70605382	Intron	C/T	0.69	-0.16	-+--	2.15E-05	9.77E-05	9.57E-04
rs750764	SLC4A3	2	220489250	3'	C/T	0.52	-0.16	-----	2.34E-05	9.10E-04	1.65E-02
rs3805383	NMU	4	56170095	Coding	G/A	0.25	-0.17	-----	2.35E-05	1.08E-03	6.28E-03
rs1007684	SLC4A3	2	220498881	3'	G/A	0.48	0.15	++++	2.52E-05	1.08E-03	1.13E-02
rs7926934	ABCC8	11	17389664	Intron	C/A	0.91	0.26	++++	2.64E-05	2.13E-03	1.13E-02
rs3010301	CITED2	6	139816546	5'	C/T	0.74	0.17	++++	2.65E-05	5.25E-04	1.36E-02
rs4234121	KIF1A	2	241417954	5'	C/A	0.57	-0.15	-----	3.25E-05	9.26E-03	5.32E-05
rs7102022	CNTN5	11	97988412	5'	G/A	0.57	0.15	++++	3.29E-05	2.40E-04	1.93E-02
rs11253779	C10orf97	10	16178495	5'	C/T	0.68	-0.16	-----	3.36E-05	7.26E-02	4.51E-04
rs388159	IL12RB1	19	18002996	3'	C/T	0.18	0.19	++++	3.47E-05	2.06E-05	7.31E-02
rs4148634	ABCC8	11	17386448	Intron	C/T	0.09	-0.26	-+--	3.48E-05	3.08E-03	1.31E-02
rs7636840	IL12A	3	161161335	5'	G/T	0.17	-0.19	-----	3.58E-05	1.45E-03	3.20E-02
rs4856360	ROBO1	3	79971091	5'	G/A	0.55	0.15	++++	4.07E-05	1.70E-03	2.05E-02
rs1453488	LPIN1	2	12284359	3'	C/T	0.80	0.18	++++	4.35E-05	4.36E-03	1.54E-03
rs9848056	ROBO1	3	79967365	5'	C/T	0.46	-0.15	-----	4.67E-05	1.60E-03	2.26E-02
rs10763141	PCDH15	10	56187416	Intron	G/A	0.24	-0.17	-----	4.71E-05	2.21E-03	2.78E-02
rs6788848	ZPLD1	3	104510935	3'	C/T	0.78	-0.18	-----	4.99E-05	4.28E-04	8.57E-03
rs10795348	C10orf97	10	16198886	5'	C/T	0.68	-0.16	-----	5.07E-05	6.63E-02	3.88E-04
rs7098374	C10orf97	10	16185793	5'	C/T	0.31	0.16	++++	5.11E-05	1.12E-01	1.82E-04
rs4669834	LPIN1	2	12287321	3'	G/A	0.80	0.18	++++	5.13E-05	6.32E-03	1.60E-03
rs1843593	GNRHR	4	68281593	3'	C/T	0.85	-0.20	-----	5.30E-05	5.37E-04	1.52E-01
rs1321267	MOXD1	6	132508145	3'	C/A	0.48	-0.14	-----	5.46E-05	1.66E-03	4.55E-03
rs11918254	IL12A	3	161152688	5'	C/T	0.17	-0.19	-----	6.29E-05	2.04E-03	4.46E-02
rs4651208	C1orf21	1	182708677	Intron	C/T	0.68	-0.15	-----	6.34E-05	2.02E-02	2.71E-03
rs4735916	ERICH1	8	667305	Intron	G/A	0.10	-0.24	-----	7.02E-05	1.16E-02	3.11E-03
rs561553	FLJ12681	16	835080	3'	C/A	0.42	0.15	++++	7.11E-05	5.04E-02	2.61E-04
rs2063485	ZPLD1	3	104831389	3'	C/T	0.88	0.23	++++	7.18E-05	3.54E-02	1.34E-05
rs7434819	C4orf18	4	158950469	3'	G/A	0.39	-0.14	-----	7.34E-05	1.09E-02	9.32E-04
rs925847	STAT4	2	191605785	Intron	C/T	0.28	0.16	++++	8.17E-05	5.65E-03	5.22E-04
rs4984698	FLJ12681	16	837567	3'	G/A	0.17	-0.19	-----	8.33E-05	3.27E-03	5.74E-05
rs9903394	SOX9	17	67603028	5'	G/A	0.77	0.16	++++	8.47E-05	1.12E-03	6.28E-03
rs3756089	IRF2	4	185552786	Intron	C/T	0.08	0.26	++++	8.79E-05	3.78E-03	7.70E-03
rs7670758	HHIP	4	145731325	5'	G/A	0.44	-0.14	-----	9.50E-05	1.70E-03	6.21E-02
rs285461	LRRCS2	1	163764316	5'	C/T	0.13	0.22	++++	9.56E-05	4.18E-03	3.29E-03
rs727755	C4orf18	4	158944298	3'	G/A	0.38	-0.14	-----	9.57E-05	1.25E-02	2.98E-03
rs10951730	HECW1	7	43400395	Intron	G/A	0.10	-0.24	-+--	9.58E-05	2.78E-04	2.07E-03
rs10892026	CNTN5	11	97979933	5'	G/A	0.43	-0.14	-----	9.83E-05	7.75E-04	2.76E-02
rs9364299	SMOC2	6	168944777	3'	G/A	0.37	0.14	++++	9.96E-05	3.74E-04	5.55E-02

TABLE E3. Mean ppFEV₁ values for 4 associated T_H1 pathway SNPs in SARP

SNP	Gene	Genotype	Counts/frequency	ppFEV ₁
rs7636840	<i>IL12A</i>	TT	21/0.034	60.0 ± 19.0
		TG	175/0.28	75.2 ± 22.7
		GG	422/0.68	77.6 ± 20.8
rs388159	<i>IL12RB1</i>	TT	23/0.037	85.1 ± 21.5
		TC	178/0.29	78.8 ± 20.2
		CC	417/0.67	74.8 ± 21.9
rs925847	<i>STAT4</i>	TT	44/0.071	81.1 ± 19.3
		TC	241/0.39	77.5 ± 22.1
		CC	331/0.54	74.9 ± 21.3
rs3756089	<i>IRF2</i>	TT	2/0.0032	83.5 ± 5.0
		TC	82/0.13	82.4 ± 22.3
		CC	534/0.86	75.4 ± 21.3

TABLE E4. Meta-analysis results of the top 39 SNPs ($P < 10^{-4}$) associated with ppFVC values

SNP	Gene	Chromosome	BP	Location	Ref/Alt allele	Alt freq	Alt effect	Direction	ppFVC	ppFEV ₁	FEV ₁ /FVC ratio
rs12984174	<i>IL12RB1</i>	19	17993540	3'	C/T	0.87	-0.26	---	8.89E-06	6.74E-04	7.64E-01
rs12437784	<i>MCTP2</i>	15	93288056	3'	G/A	0.40	0.18	+++	1.01E-05	1.74E-02	5.40E-01
rs4752536	<i>FGFR2</i>	10	123082797	3'	G/A	0.34	-0.18	---	1.06E-05	4.66E-03	1.89E-01
rs9827557	<i>NLGN1</i>	3	175761334	3'	G/A	0.79	-0.22	---	1.12E-05	2.36E-03	2.74E-01
rs11199916	<i>FGFR2</i>	10	123084851	3'	C/A	0.66	0.18	+++	1.37E-05	4.44E-03	1.78E-01
rs17592868	<i>YTHDC1</i>	4	68897521	Intron	C/T	0.93	0.35	+++	1.39E-05	2.54E-02	9.70E-01
rs2121743	<i>DNHD2</i>	3	57407025	5'	C/T	0.54	0.17	+++	1.56E-05	1.37E-03	3.56E-01
rs13119846	<i>KIAA0922</i>	4	154634634	Intron	C/T	0.54	-0.17	---	2.02E-05	9.07E-03	7.86E-01
rs388159	<i>IL12RB1</i>	19	18002996	3'	C/T	0.18	0.22	+++	2.06E-05	3.47E-05	7.31E-02
rs950636	<i>NAALADL2</i>	3	175782739	5'	G/A	0.83	-0.22	---	2.32E-05	1.93E-03	2.92E-01
rs221013	<i>PAK7</i>	20	9763528	Intron	C/T	0.36	0.17	+++	3.09E-05	2.31E-03	1.71E-01
rs4133045	<i>TNIF</i>	3	172251865	3'	G/A	0.23	0.20	+++	3.50E-05	2.20E-03	6.77E-01
rs6496069	<i>MCTP2</i>	15	93272064	3'	C/T	0.31	0.18	+++	3.54E-05	5.13E-03	4.91E-01
rs4360694	<i>USP47</i>	11	11875274	Intron	C/T	0.68	0.18	+++	3.73E-05	1.18E-03	3.51E-02
rs17231818	<i>PLXNC1</i>	12	93026715	5'	C/T	0.84	0.23	+++	3.94E-05	3.42E-03	1.71E-01
rs4681992	<i>FLJ44290</i>	3	57464631	Intron	C/A	0.54	0.16	+++	4.01E-05	2.26E-03	3.54E-01
rs169660	<i>HS3ST2</i>	16	22748205	Intron	C/T	0.37	-0.17	---	4.20E-05	2.50E-03	6.81E-02
rs4910031	<i>USP47</i>	11	11878901	Intron	C/T	0.51	0.16	+++	4.54E-05	3.26E-04	1.77E-02
rs2497714	<i>ABLIM1</i>	10	116282551	Intron	G/A	0.06	0.33	+++	4.66E-05	2.11E-02	5.45E-01
rs7308292	<i>PLXNC1</i>	12	93024721	5'	G/A	0.22	-0.20	---	4.67E-05	6.47E-04	4.65E-03
rs11085898	<i>NDUFB7</i>	19	14540428	Intron	G/A	0.45	0.16	+++	4.97E-05	7.67E-04	2.39E-02
rs1291183	<i>YES1</i>	18	839178	5'	G/T	0.29	-0.18	---	5.47E-05	3.54E-06	1.18E-02
rs13187105	<i>PPP2CA</i>	5	133579344	Intron	G/T	0.18	-0.21	---	5.54E-05	4.41E-03	7.87E-01
rs6096573	<i>ATP9A</i>	20	49817086	Intron	C/T	0.47	0.16	+++	5.92E-05	1.30E-04	6.07E-03
rs2110565	<i>BCL11A</i>	2	59602905	3'	C/T	0.55	-0.16	---	6.15E-05	1.24E-02	4.24E-01
rs10466868	<i>LOC338797</i>	12	130505873	3'	G/T	0.12	-0.25	---	6.39E-05	8.75E-03	3.32E-01
rs6482071	<i>PLXDC2</i>	10	20269755	Intron	C/T	0.07	0.31	+++	6.50E-05	5.03E-04	6.28E-02
rs2181563	<i>KIAA1600</i>	10	116594334	Intron	G/A	0.52	-0.17	---	6.72E-05	4.07E-03	4.84E-01
rs7281703	<i>C21orf37</i>	21	17374096	5'	G/T	0.07	0.33	+++	6.98E-05	3.99E-02	8.34E-01
rs8115491	<i>PTPRT</i>	20	40280900	Intron	C/T	0.80	0.20	+++	7.02E-05	6.33E-04	3.31E-01
rs6500728	<i>A2BP1</i>	16	5840890	5'	C/T	0.33	-0.17	---	7.15E-05	3.27E-04	2.17E-01
rs10051026	<i>PPP2CA</i>	5	133559348	3'	C/T	0.82	0.21	+++	7.16E-05	6.04E-03	7.71E-01
rs1208082	<i>ALS2CR4</i>	2	202205831	Intron	G/T	0.29	-0.17	---	8.49E-05	4.29E-03	6.70E-01
rs9974012	<i>BMP7</i>	20	54966696	3'	C/T	0.40	0.16	+++	8.72E-05	2.74E-02	5.83E-01
rs1822934	<i>LOC119710</i>	11	37395924	3'	G/A	0.44	-0.16	---	9.35E-05	2.07E-02	3.57E-01
rs1822933	<i>LOC119710</i>	11	37353037	3'	G/A	0.44	-0.16	---	9.49E-05	1.17E-02	3.80E-01
rs7836170	<i>SULF1</i>	8	70605382	Intron	C/T	0.69	-0.17	++-	9.77E-05	2.15E-05	9.57E-04
rs8140240	<i>EIF3S7</i>	22	35249289	Intron	G/A	0.93	-0.31	---	9.95E-05	4.61E-04	1.73E-01
rs7262414	<i>PTPRT</i>	20	40245194	Intron	C/A	0.20	-0.19	---	9.97E-05	1.01E-03	3.75E-01

TABLE E5. Meta-analysis results of the top 31 SNPs ($P < 10^{-4}$) associated with FEV₁/FVC ratio values

SNP	Gene	Chromosome	BP	Location	Ref/alt allele	Alt freq	Alt effect	Direction	FEV ₁ /FVC ratio	ppFEV ₁	ppFVC
rs2063485	<i>ZPLD1</i>	3	104831389	3'	C/T	0.88	0.25	++++	1.34E-05	7.18E-05	3.54E-02
rs5767064	<i>LOC388915</i>	22	46985678	3'	G/A	0.61	0.16	++++	2.79E-05	2.60E-03	3.69E-01
rs11032873	<i>APIP</i>	11	34763042	3'	C/T	0.78	0.18	++++	3.08E-05	3.05E-03	7.00E-01
rs17646998	<i>SULF1</i>	8	70646715	Intron	C/T	0.37	-0.15	----	3.61E-05	1.32E-02	9.76E-01
rs823673	<i>NFYC</i>	1	41013106	3'	G/A	0.81	0.19	++++	3.94E-05	3.45E-04	1.11E-01
rs4234121	<i>KIF1A</i>	2	241417954	5'	C/A	0.57	-0.15	----	5.32E-05	3.25E-05	9.26E-03
rs3809335	<i>MTMR6</i>	13	24759577	5UTR	C/T	0.93	0.28	++++	5.40E-05	2.96E-03	2.05E-01
rs556179	<i>FLJ12681</i>	16	825033	3'	G/A	0.52	0.15	+++	5.43E-05	3.65E-04	1.86E-01
rs7663065	<i>FLJ45721</i>	4	28296504	5'	G/A	0.67	-0.15	----	5.43E-05	3.14E-02	6.92E-01
rs12659620	<i>ADCY2</i>	5	7208958	5'	C/T	0.54	0.15	++++	5.56E-05	1.84E-03	4.43E-01
rs4984698	<i>FLJ12681</i>	16	837567	3'	G/A	0.17	-0.20	----	5.74E-05	8.33E-05	3.27E-03
rs9287995	<i>HNRPA3</i>	2	177592478	5'	C/T	0.42	0.15	++++	6.02E-05	9.33E-03	1.27E-01
rs104664	<i>C22orf8</i>	22	44090518	Intron	G/A	0.87	-0.22	----	6.14E-05	3.47E-03	9.89E-02
rs470062	<i>SMC1L2</i>	22	44146033	Coding	G/A	0.87	-0.22	----	6.21E-05	2.99E-03	1.03E-01
rs2230739	<i>ADCY9</i>	16	3973437	Coding	G/A	0.69	0.16	++++	6.25E-05	5.20E-04	7.81E-02
rs3827393	<i>C22orf8</i>	22	44114817	Intron	G/A	0.88	-0.22	----	7.02E-05	3.45E-03	1.17E-01
rs11675728	<i>DNER</i>	2	230034312	Intron	C/T	0.43	-0.15	----	7.22E-05	2.23E-02	6.75E-01
rs6006992	<i>SMC1L2</i>	22	44121424	Intron	G/A	0.12	0.22	++++	7.24E-05	3.49E-03	1.18E-01
rs3130696	<i>HLA-C</i>	6	31351863	5'	G/A	0.25	-0.17	----	7.34E-05	5.23E-03	3.01E-01
rs689439	<i>LOC388199</i>	16	815403	3'	G/A	0.49	0.14	+++	7.39E-05	2.19E-04	7.33E-02
rs1656941	<i>RFC4</i>	3	188001835	Intron	C/A	0.79	0.17	+++	7.44E-05	5.12E-03	1.89E-01
rs2705044	<i>MTMR7</i>	8	17366532	5'	C/A	0.87	0.21	++++	7.46E-05	2.80E-02	6.71E-01
rs1406593	<i>SNX10</i>	7	26394414	3'	C/T	0.35	-0.15	----	7.64E-05	3.73E-02	9.24E-01
rs9574386	<i>C13orf10</i>	13	78732483	3'	G/A	0.08	-0.27	----	7.72E-05	2.81E-04	7.81E-02
rs8030494	<i>TBC1D21</i>	15	71879830	5'	C/A	0.63	0.15	++++	7.83E-05	3.50E-03	5.04E-01
rs1648703	<i>RFC4</i>	3	188001830	Intron	G/T	0.79	0.17	+++	7.83E-05	5.22E-03	1.91E-01
rs3748540	<i>GREM2</i>	1	238760334	Intron	G/A	0.65	0.15	++++	7.88E-05	4.45E-03	1.02E-02
rs17554448	<i>ZNF650</i>	2	170463100	5'	G/A	0.83	0.19	++++	8.56E-05	1.64E-02	4.97E-01
rs9362054	<i>C6orf84</i>	6	85234987	5'	C/T	0.46	0.14	++++	8.68E-05	1.51E-01	2.30E-01
rs17450685	<i>C10orf11</i>	10	77776943	Intron	C/T	0.37	0.15	++++	8.73E-05	1.60E-03	1.01E-01
rs1416920	<i>ZNF323</i>	6	28410763	Intron	C/T	0.73	0.16	++++	9.46E-05	1.59E-03	1.80E-01

TABLE E6. Replication of 12 lung function loci identified in the general population

Previously identified genes: SNPs	Tested SNPs	LD	Minor/risk allele	ppFEV ₁	ppFVC	FEV ₁ /FVC ratio
<i>HHIP</i> : rs1980057	rs1980057	1	T/C	0.0017	0.016	0.12
	rs7670758	0.59	A/A	0.000095	0.0017	0.013
<i>GPR126</i> : rs11155242	rs11155242	1	C/C?	0.78	0.29	0.35
	rs7745188	0.006	G/G	0.27	0.0091	0.66
<i>ADAM19</i> : rs2277027	rs2277027	1	C/C?	0.4	0.59	0.43
	rs17054692	0	G/G	0.14	0.85	0.0091
<i>NOTCH4-AGER</i> : rs2070600	rs206015	0.65	T/T	0.064	0.032	0.55
	rs2849015	0.023	A/G	0.0023	0.22	0.0007
<i>FAM13A</i> : rs2869967	rs2869967	1	C/C	0.075	0.058	0.085
	rs6825998	0.23	T/C	0.013	0.023	0.08
<i>PTCH</i> : rs16909898	rs16909898	1	G/A?	0.69	0.23	0.45
	rs576594	0.087	T/T	0.0082	0.026	0.079
<i>PIDI</i> : rs1435867	rs1435867	1	C/T?	0.13	0.74	0.061
	rs10211472	0.018	T/T	0.0065	0.028	0.039
<i>HTR4</i> : rs3995090	rs7733088	0.93	A/G?	0.66	0.18	0.37
	rs17720733	0.005	C/C	0.02	0.07	0.067
<i>INTS12-GSTCD-NPNT</i> : rs10516526	rs17035917	0.88	T/C	0.33	0.0099	0.51
	rs6820671	0.67	G/A	0.025	0.0019	0.15
<i>TNSI</i> : rs2571445	rs2571445	1	T/C?	0.49	0.63	0.25
	rs3791890	0.001	C/T	0.024	0.058	0.039
<i>THSD4</i> : rs12899618	rs12899618	1	A/A	0.32	0.53	0.034
	rs12917055	0.012	A/G	0.026	0.97	0.0019
<i>DAAM2</i> : rs2395730	rs2395730	1	C/A?	0.53	0.69	0.53
	rs3793137	0	A/C	0.099	0.78	0.0022

?, Uncertainty of risk allele.

TABLE E7. Replication of 16 lung function loci identified in the general population

Previously identified genes: SNPs	Tested SNPs	LD	Minor/risk allele	ppFEV ₁	ppFVC	FEV ₁ /FVC ratio
<i>MFAP2</i> : rs2284746	rs2871775	0.66	T/C?	0.87	0.21	0.54
	rs2647168	0.02	A/G?	0.11	0.32	0.08
<i>TGFB2</i> : rs993925	rs11583395	0.58	G/G?	0.62	0.71	0.11
	rs4846479	0.005	T/G	0.21	0.91	0.0071
<i>HDAC4</i> : rs12477314	rs4591362	0.94	A/G?	0.55	0.26	0.39
	rs7599854	0.051	T/C	0.054	0.48	0.0067
<i>RARB</i> : rs1529672	rs1529672	1	A/C	0.32	0.39	0.0038
	rs7616278	0.23	A/G	0.019	0.34	0.00039
<i>MECOM</i> : rs1344555	rs1362772	1	T/C?	0.52	0.9	0.34
	rs988397	0.007	T/T	0.047	0.14	0.045
<i>SPATA9</i> : rs153916	rs2548125	0.61	C/C?	0.4	0.12	0.99
	rs2158095	0.14	T/C?	0.68	0.36	0.25
<i>ZKSCAN3-ZNF323</i> : rs6903823	rs6922111	0.95	T/T	0.01	0.37	0.0013
	rs1416920	0.63	C/C	0.0016	0.18	0.000095
<i>NCR3</i> : rs2857595	rs2857595	1	T/T?	0.15	0.1	0.39
	rs1800629	0.86	A/A	0.043	0.058	0.078
<i>ARMC2</i> : rs2798641	rs2798641	1	T/T?	0.77	0.49	0.092
	rs13219800	0.08	G/A	0.39	0.21	0.052
<i>CDC123</i> : rs7068966	rs1051055	0.59	A/G?	0.55	0.32	0.99
	rs7895410	0.36	C/C	0.18	0.63	0.022
<i>C10orf11</i> : rs11001819	rs1907323	0.73	T/T?	0.17	0.35	0.16
	rs17450685	0.049	T/C	0.0016	0.11	0.000087
<i>LRP1</i> : rs11172113	rs11172113	1	C/C?	0.91	0.59	0.93
	rs1799986	0.003	T/C	0.03	0.053	0.04
<i>CCDC38</i> : rs1036429	rs1036429	1	T/C?	0.43	0.68	0.073
	rs1436124	0.7	A/C	0.17	0.37	0.027
<i>MMP15</i> : rs12447804	rs12929002	0.96	A/A?	0.73	0.42	0.16
	rs11648508	0.48	G/G?	0.35	0.39	0.16
<i>CFDP1</i> : rs2865531	rs977987	0.76	C/T?	0.65	0.08	0.69
	rs37602	0.23	T/T	0.0048	0.0012	0.0094
<i>KCNE2</i> : rs9978142	rs7278204	0.81	G/G?	0.33	0.27	0.79
	rs8127788	0.19	G/G	0.018	0.12	0.058

?, Uncertainty of risk allele.

TABLE E8. Four T_H1 pathway SNPs are not associated with asthma susceptibility

SNP	Gene	Chromosome	BP	Location	P value		
					SARP/CSGA	TENOR	GABRIEL*
rs7636840	<i>IL12A</i>	3	161161335	5'	.85	.7	.7
rs388159	<i>IL12RB1</i>	19	18002996	3'	.015	.34	.48 (rs12984174)†
rs925847	<i>STAT4</i>	2	191605785	Intron	.11	.13	.44
rs3756089	<i>IRF2</i>	4	185552786	Intron	.087	.55	.69

*Extracted from the European Genome-Phenome Archive; <http://www.cng.fr/gabriel>; accession no. EGAS00000000077.

†rs12984174 is in moderate LD with rs388159 ($r^2 = 0.75$).

TABLE E9. Six SNPs conferring asthma susceptibility are not associated with ppFEV₁ values

SNP	Gene	Chromosome	BP	Location	Ref/alt allele	Alt freq	Alt effect	Direction	ppFEV ₁
rs2872507	<i>ORMDL3-GSDMB</i>	17	35294289	3'	G/A	0.42	0.0066	+++−	0.86
rs3939286	<i>IL33</i>	9	6200099	5'	G/A	0.28	−0.0135	+−+−	0.74
rs3771166	<i>IL1RL1-IL18R1</i>	2	102352654	Intron	C/T	0.35	−0.0069	−+−+	0.85
rs1837253	<i>TSLP</i>	5	110429771	5'	C/T	0.22	−0.0319	+−−−	0.46
rs20541	<i>IL13</i>	5	132023863	Coding	C/T	0.22	−0.0219	++−−	0.61
rs2395185	<i>HLA-DRA</i>	6	32541145	3'	G/T	0.37	−0.0773	−−−−	0.043

TABLE E10. Combinations of risk alleles in 4 T_H1 pathway genes with ppFEV₁ values

No. of risk alleles	Combination	n	ppFEV ₁
1	<i>IRF2</i> het	1	96
2	<i>IRF2</i> homo	1	107
	<i>IRF2</i> het + <i>IL12RB1</i> het	1	91
3	<i>IRF2</i> homo + <i>IL12RB1</i> het	7	82.1 ± 20.9
	<i>IRF2</i> homo + <i>STAT4</i> het	8	84.6 ± 24.3
	<i>IRF2</i> homo + <i>IL12A</i> het	2	100.0 ± 9.9
	<i>IRF2</i> het + <i>IL12RB1</i> homo	3	78.7 ± 22.0
	<i>IRF2</i> het + <i>IL12RB1</i> het + <i>STAT4</i> het	6	92.3 ± 14.9
4	<i>IRF2</i> homo + <i>IL12RB1</i> homo	19	78.2 ± 19.9
	<i>IRF2</i> homo + <i>IL12RB1</i> het + <i>STAT4</i> het	43	80.2 ± 20.2
	<i>IRF2</i> homo + <i>IL12RB1</i> het + <i>IL12A</i> het	4	69.8 ± 23.3
	<i>IRF2</i> homo + <i>STAT4</i> homo	5	75.6 ± 15.0
	<i>IRF2</i> het + <i>IL12RB1</i> homo + <i>STAT4</i> het	20	78.9 ± 24.0
	<i>IRF2</i> het + <i>IL12RB1</i> homo + <i>IL12A</i> het	2	96.5 ± 14.8
	<i>IRF2</i> het + <i>IL12RB1</i> het + <i>STAT4</i> homo	6	88.7 ± 12.8
	<i>IRF2</i> het + <i>IL12RB1</i> het + <i>STAT4</i> het + <i>IL12A</i> het	5	89.6 ± 22.0
	<i>IRF2</i> het + <i>STAT4</i> homo + <i>IL12A</i> het	1	90
	<i>STAT4</i> homo + <i>IL12RB1</i> homo	2	83.5 ± 4.9
5	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>STAT4</i> het	87	76.0 ± 22.4
	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>IL12A</i> het	3	77.7 ± 14.4
	<i>IRF2</i> homo + <i>IL12RB1</i> het + <i>STAT4</i> homo	52	76.7 ± 20.4
	<i>IRF2</i> homo + <i>IL12RB1</i> het + <i>STAT4</i> het + <i>IL12A</i> het	19	77.5 ± 22.0
	<i>IRF2</i> homo + <i>STAT4</i> homo + <i>IL12A</i> het	5	82.0 ± 29.2
	<i>IRF2</i> het + <i>IL12RB1</i> homo + <i>STAT4</i> homo	20	76.6 ± 26.1
	<i>IRF2</i> het + <i>IL12RB1</i> homo + <i>STAT4</i> het + <i>IL12A</i> het	6	71.2 ± 30.6
	<i>IRF2</i> het + <i>IL12RB1</i> het + <i>STAT4</i> homo + <i>IL12A</i> het	3	89.3 ± 18.8
	<i>IRF2</i> het + <i>IL12RB1</i> het + <i>STAT4</i> het + <i>IL12A</i> homo	1	73
6	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>STAT4</i> homo	140	75.7 ± 19.8
	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>STAT4</i> het + <i>IL12A</i> het	39	76.1 ± 22.2
	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>IL12A</i> homo	1	74
	<i>IRF2</i> homo + <i>IL12RB1</i> het + <i>STAT4</i> homo + <i>IL12A</i> het	24	77.3 ± 19.1
	<i>IRF2</i> het + <i>IL12RB1</i> homo + <i>STAT4</i> homo + <i>IL12A</i> het	7	91.1 ± 17.0
7	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>STAT4</i> homo + <i>IL12A</i> het	54	67.3 ± 23.0
	<i>IRF2</i> homo + <i>IL12RB1</i> het + <i>STAT4</i> homo + <i>IL12A</i> homo	6	59.8 ± 19.9
	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>STAT4</i> het + <i>IL12A</i> homo	7	60.6 ± 17.3
8	<i>IRF2</i> homo + <i>IL12RB1</i> homo + <i>STAT4</i> homo + <i>IL12A</i> homo	6	55.0 ± 24.1

het, Heterozygote for risk allele; homo, homozygote for risk allele.