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Importance of hedgehog interacting protein and other lung function genes in asthma

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Abstract

Background—Two recent large meta-analyses of genome-wide association studies of lung function in general populations of European descent identified 11 candidate genes/regions. The importance of these genes in lung function in whites and African Americans with asthma is unknown.

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Clinical Implications Lung function is a major determinant of asthma severity. Combinations of risk lung function SNPs in *HHIP*, *PTCH1*, and *FAM13A* identified asthmatics with lower levels of lung function.

Capsule summary Our results show that specific genes affect not only normal variations in lung function but importantly, also level of lung function in asthma, and may be useful in predicting lung function decline and asthma severity.

Objectives—To determine if genes that regulate lung function in general populations are associated with lung function abnormalities in subjects with asthma from different racial groups.

Methods—SNPs were tested in five asthma populations (n = 1,441) for association with pulmonary function and meta-analysis was performed across populations. The SNPs with the highest significance were then tested for association with bronchodilator reversibility and bronchial hyperresponsiveness to methacholine (BHR). A joint analysis of consistently replicated SNPs was performed to predict lung function in asthma.

Results—Hedgehog interacting protein (*HHIP*) on chromosome 4q31 was associated with lung function in all five populations, rs1512288: $P_{\text{meta}} = 9.62\text{E-}05$ and $3.23\text{E-}05$ for ppFEV₁ and ppFVC, respectively. The SNPs in *HHIP* were also associated with reversibility ($P < 0.05$) but not BHR. Because of differences in linkage disequilibrium in the African-American subjects, the most relevant SNPs in *HHIP* were identified. A subset of normal lung function genes, including *HHIP*, family with sequence similarity 13, member A (*FAM13A*), and patched homolog 1 (*PTCH1*), together predict lung function abnormalities, a measure of severity in whites and African Americans with asthma.

Conclusion—A subset of the genes, including *HHIP*, which regulate lung function in general populations are associated with abnormal lung function in asthma in non-Hispanic whites and African Americans.

Keywords

Asthma; Genetics; Asthma severity; Meta-analysis; FEV₁; FVC; FEV₁/FVC; *HHIP*; *FAM13A*; *PTCH1*

INTRODUCTION

Asthma is a heterogeneous disease which is classified phenotypically as mild, moderate, or severe based on NAEPP, GINA, or ATS guidelines¹⁻³ and more recently, five asthma severity phenotypes were identified using cluster analysis.⁴ The majority of morbidity and health care utilization occurs in severe asthma; a phenotype largely determined by pulmonary function (baseline lung function: percent predicted (pp) FEV₁, ppFVC, and FEV₁/FVC).^{3,4} Quantitative phenotypes such as ppFEV₁ are essential for categorizing asthma severity for both current guidelines classification or with cluster methodologies.⁵

A recent GWAS meta-analysis for pulmonary function in 20,890 participants from general populations of European white ancestry (CHARGE consortium) found that genes in the *INTS12-GSTCD-NPNT* region were associated with FEV₁ and eight genes (*HHIP*, *GPR126*, *ADAM19*, *AGER-PPT2*, *FAM13A*, *PTCH1*, *PID1*, and *HTR4*) were associated with FEV₁/FVC.⁶ A second GWAS meta-analysis for lung function in general populations (20,288 participants of European whites ancestry: SpiroMeta consortium), identified four genes (*HHIP*, *GSTCD*, *TNSI*, and *HTR4*) associated with FEV₁ and three loci (*HHIP*, *NOTCH4-AGER-PPT2*, *THSD4*) associated with FEV₁/FVC.⁷ While these genes may only influence lung function in subjects without respiratory diseases, we hypothesize that some of these genes are important in determining lung function in subjects with asthma. Identifying the genetic variants that influence pulmonary function in asthma is of major importance because these genomic approaches will lead to improved understanding of biologic factors that regulate lung function in asthma: a fundamental determinant of asthma severity.⁸

In this study, we performed meta-analysis of ppFEV₁, ppFVC, and FEV₁/FVC in five asthma populations including non-Hispanic whites and African Americans to determine if the genes previously identified in the two GWAS for normal variation of lung function are

important in determining lung function in subjects with asthma. Our primary hypothesis was to determine whether the previously identified SNP in each gene which was significant in the general population is important in asthma; therefore we analyzed 1 SNP in each of the 11 genes (Three additional SNPs were analyzed in *HHIP* since they are in strong LD with the previously identified SNP.). In order to present a more comprehensive view of each gene, the additional SNPs already genotyped in the 11 genes were analyzed as a secondary analysis. Importantly, the previous studies did not include subjects of African descent, a population with increased frequency and severity of asthma.^{3,5,9,10}

METHODS

Study Subjects

Subjects with mild to severe asthma were recruited at the NHLBI funded Severe Asthma Research Program (SARP) centers and carefully characterized including baseline spirometry with a medication withhold before testing.^{3,4} 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) multi-center study.^{11,12} In addition, subjects with asthma who were studied in the NHLBI Collaborative Studies on the Genetics of Asthma (CSGA) by the Wake Forest investigators using similar protocol were included in the analyses.⁹ TENOR, SARP, and CSGA studies were approved by the appropriate Institutional Review Board at the participating sites including appropriate informed consent.

DNA was isolated using standard protocols, and SNP genotyping was performed using the Illumina HumanCNV370 BeadChip for TENOR.¹² SARP and CSGA samples were genotyped using the Illumina HumanHap1M BeadChip (unpublished data). Genotyping for both studies was performed using BeadStudio (Illumina, Inc.).

Statistical Analysis

SARP cases were removed if they 1) had low genotyping call rates ($< 95\%$), 2) were discrepant or ambiguous for genetic sex (heterozygous haploid genotype percentage ≥ 0.01 or X chromosome homozygosity $F \geq 0.9$), 3) were different from described race, 4) failed the check for family relatedness ($PI\_HAT > 0.125$), 5) were detected as an outlier (> 6 standard deviation for the first or second principal component). After subjects meeting these criteria were excluded, SNPs were removed if 1) the call rates were still low (95%), 2) inconsistent with Hardy-Weinberg Equilibrium (HWE) ($P < 10E-04$), 3) minor allele frequency (MAF) was less than 0.05. Quality control of TENOR and CSGA cases was performed in a similar manner.

A linear additive model was used for analysis of $ppFEV_1$, $ppFVC$, FEV_1/FVC , and gene-gene interaction analysis using PLINK (version 1.06, URL: <http://pngu.mgh.harvard.edu/purcell/plink/>),¹³ adjusted for age, sex, and significant principal components (EIGENSTRAT, version 3.0, URL: <http://genepath.med.harvard.edu/~reich/Software.htm>).¹⁴ Haploview (URL: <http://www.broad.mit.edu/mpg/haploview/>) was used to generate linkage disequilibrium plots,¹⁵ and 95% confidence intervals on D' was used to define blocks.¹⁶ For the primary analysis, 14 SNPs in 11 candidate genes/regions were analyzed for replication at SNP level (rs6845536, rs720485, rs1828591, and rs1512288 in *HHIP*, rs2571445 in *TNSI*, rs10516526 in *INTS12-GSTCD-NPNT*, rs3995090 in *HTR4*, rs2070600 in *NOTCH-AGER-PPT2*, rs1913768 in *THSD4*, rs11155242 in *GPR126*, rs2277027 in *ADAM19*, rs2869967 in *FAM13A*, rs10512249 in *PTCH1*, and rs1358443 in *PID1*). Using a more relaxed replication

standard,¹⁷ assuming gene as a unit and related phenotypes as similar, the most significant SNPs were reported for each candidate genes.

Meta-analysis of P values from three non-Hispanic white populations, two African American populations, or all of five populations was performed with weights proportional to the squareroot of the sample size using METAL software (URL:

<http://www.sph.umich.edu/csg/abecasis/metal/>). Meta-analysis of effect size (regression slope) of the identified candidate SNPs was performed with weights proportional to sample size using rmeta package based on random effects model (URL:

<http://cran.rproject.org/web/packages/rmeta/index.html>). To reduce genomic inflation, P values with genomic control adjustment from each population were used for meta-analysis.

Joint analysis of three most consistent SNPs (rs1512288 in *HHIP*, rs576594 in *PTCH1*, and rs2869967 in *FAM13A*) or five SNPs (rs1512288 in *HHIP*, rs576594 in *PTCH1*, rs2869967 in *FAM13A*, rs3845823 in *PID1*, and rs206015 in *NOTCH4*) was performed in SARP non-Hispanic whites, SARP African Americans, and TENOR whites. Genotypes with 1 or 2 minor alleles were merged together and recoded as 0 (if minor allele was protective allele) or 1 (if minor allele was risk allele). A linear additive model was used for analysis of ppFEV₁, ppFVC, FEV₁/FVC with the number of risk SNPs. A logistic regression model was used for analysis of percent of subjects with severe asthma using ATS and cluster classification^{3,4} with the number of risk SNPs.

RESULTS

Although the three studies used similar clinical approaches to carefully phenotype cases, they differ in the proportion of subjects with severe asthma since SARP is a cohort that is enriched for subjects with severe asthma; TENOR focused on “difficult-to-treat” or severe asthma; while CSGA recruited a cohort including all levels of severity but primarily focused on milder levels of asthma severity. These differences are reflected in baseline lung function (see Table E1 in the Online Repository). The cohorts have a broad range of lung function with slightly lower levels in the SARP and TENOR subjects with asthma consistent with the recruitment of subjects with more severe asthma in these studies.

After quality control analysis was completed as described above, data from 1,441 subjects with asthma (SARP non-Hispanic whites (n = 438), TENOR non-Hispanic whites (n = 431), CSGA non-Hispanic whites (n = 230), SARP African Americans (n = 206), and CSGA African Americans (n = 136)) were analyzed for association with spirometric measures of lung function: ppFEV₁, ppFVC, and FEV₁/FVC, and the SNPs in 11 genes identified through two recent meta-analyses of GWAS of normal lung function. The issue of multiple testing was minimized since only 14 SNPs in 11 previously identified genes were analyzed for replication at SNP level (P value for Bonferroni adjustment was 3.57E-03, P = 0.05/14SNPs).

The SNP rs1512288 at 5' flanking region of *HHIP* showed the most consistent association in all five cohorts of subjects with asthma ($P_{\text{meta}} = 9.62\text{E-}05$, $3.23\text{E-}05$, and 0.11 for ppFEV₁, ppFVC, and FEV₁/FVC, respectively) (Table I, Fig 1A, and see Table E2 and Table E3 in the Online Repository). The linkage disequilibrium (LD) structure of *HHIP* region is different between non-Hispanic Whites and African Americans (Fig 1B and Fig 1C). The SNPs with the lowest P values: rs6845536, rs720485, rs1828591, and rs1512288, are in strong LD in non-Hispanic whites ($R^2 > 0.5$), but in weak LD in African Americans ($R^2 < 0.5$). SNP rs6845536 at 5' flanking region of *HHIP*, in LD with rs1512288 ($R^2 = 0.63$), showed the strongest signal in non-Hispanic white populations ($P_{\text{meta}} = 1.08\text{E-}04$, $3.20\text{E-}04$, and 0.08 for ppFEV₁, ppFVC, and FEV₁/FVC, respectively), while SNP rs1512288 had the

strongest signal in African Americans ($P_{\text{meta}} = 7.74\text{E-}04$, $5.11\text{E-}04$, and 0.30 for ppFEV₁, ppFVC, and FEV₁/FVC, respectively). Thus, we conclude based on the LD structure in African Americans that rs1512288 is either the causal SNP or in LD with a smaller set of SNPs (Table I and Fig 1).

The effect size (regression slope) of rs1512288 was consistent among five asthma populations (Heterogeneity P value = 0.41) (Fig 2). The summary effect size was 2.83 (with $SD = 1.37$) and ppFEV₁ increases 2.83% on average with every copy of the minor allele T. For non-Hispanic whites from the SARP cohort, the effect size was 2.32 (with $SD = 1.30$) for ppFEV₁ or 0.085 (with $SD = 0.056$) for baseline FEV₁ and FEV₁ differed by 85 ml on average with every copy of the minor allele T; for African Americans from SARP, the effect size was 5.87 (with $SD = 1.87$) for ppFEV₁ or 0.252 (with $SD = 0.077$) for baseline FEV₁ and FEV₁ differed 252 ml on average with every copy of the minor allele T.

SNPs in *HHIP* were associated with bronchodilator reversibility (rs720485: $P = 0.024$ and 0.057 for non-Hispanic whites and African Americans, respectively) but not with bronchial hyperresponsiveness to methacholine (BHR, logPC20) (see Table E4 in the Online Repository). Analysis results from the other genes previously identified for lung function in general populations identified five genes (*FAM13A*, *NOTCH4*, *THSD4*, *PTCH1*, and *PID1*) that were associated significantly with lung function in asthma at the SNP or gene level (Table II and see Table E5 and Table E6 in the Online Repository). At the SNP level, rs2869967, in the intron of family with sequence similarity 13, member A (*FAM13A*) on chromosome 4q22 was significant ($P_{\text{meta}} = 2.9\text{E-}03$ for FVC) using strict replication standards and after correction for multiple testing. This signal was driven mainly by non-Hispanic whites. *NOTCH4* on chromosome 6p21, *THSD4* on chromosome 15q23, *PTCH1* on chromosome 9q22-q31, and *PID1* on chromosome 2q36 were significant (Table II), using a more relaxed replication standard at the gene level. *TNSI* on chromosome 2q35-q36, *GSTCD* on chromosome 4q24, *HTR4* on chromosome 5q31-q33, *GPR126* on chromosome 6q23-q24, and *ADAM19* on chromosome 5q33, were not significant after correcting for multiple testing.

Among these 11 genes, *PTCH1* is another gene involved in HH signaling pathway. SNP rs576594 was associated with ppFEV₁ ($P_{\text{meta}} = 6.5\text{E-}07$), ppFVC ($P_{\text{meta}} = 1.9\text{E-}05$), and FEV₁/FVC ($P_{\text{meta}} = 1.1\text{E-}03$) in the white populations, specifically the extensively phenotyped SARP cohort with broader ranges of asthma severity and lung function (Table II and see Table E5 and Table E6 in the Online Repository). The association of rs576594 was observed in the African Americans, although weaker ($P < 0.05$) and interestingly in the opposite direction. Since *HHIP* and *PTCH1* had overlapping functions in HH signaling pathway, gene-gene interaction between them was analyzed. Interactions were tested between rs1512288 of *HHIP* and 14 *PTCH1* SNPs ($LD R^2 < 0.3$). No interaction was found in three white populations, however interaction ($P < 0.05$) was observed in the two African American populations (rs3824491: $P = 8.5\text{E-}03$, 0.071 , and 0.051 for meta-analysis, SARP, and CGSA African Americans, respectively) (see Table E7 in the Online Repository). This interaction needs to be replicated in other populations since the two African American populations have smaller sample sizes.

Joint analysis of the most consistently associated SNPs, based on our/previous study and biological function^{6,7} (rs1512288 in *HHIP*, rs576594 in *PTCH1*, and rs2869967 in *FAM13A*), was performed. With the increase in the number of risk SNPs, ppFEV₁, ppFVC, and FEV₁/FVC decreased (range of p values of 0.08 to 0.003) (Table III). The results were consistent for SARP non-Hispanic whites, SARP African Americans and TENOR, and may be generalized to other populations. Modeling with the three best SNPs, limited variance ($0.7\% - 4\%$) was explained (Table III), indicating there are maybe more genes involved.

Joint analysis of SNPs from 5 replicated genes (*HHIP*, *PTCH1*, *FAM13A*, *PID1*, and *NOTCH4*) generated a more significant trend in SARP non-Hispanic whites, a cohort that was recruited for a wide range of asthma severity and pulmonary function ($P = 9.8\text{E-}06$ for ppFEV₁) (Fig 3A and see Table E8 in the Online Repository), and less significant in TENOR, a cohort with a narrower range of lung function ($P = 0.01$ for ppFEV₁). In SARP non-Hispanic whites, ppFEV₁ decreased significantly from 85.4 to 80.8, then to 78.5, 74.5, and finally to 62.9 when the number of risk SNPs increased from 0 to 5 (Fig 3A). Because of this relationship, we analyzed two measures of asthma severity in the SARP non-Hispanic whites. The first was based on ATS criteria for severe asthma³ by comparing severe asthma (group 5) and mild asthma (groups 1 and 2) while the second used our newer approach to asthma phenotypic classification based on cluster analysis⁴ by comparing the more severe clusters (clusters 3, 4, and 5) with the less severe clusters (clusters 1 and 2). As seen in Figure 3B, there is an increase in severity as the number of risk SNPs increases ($P = 6.0\text{E-}04$ and $2.7\text{E-}03$ for ATS and cluster classification, respectively).

DISCUSSION

In this study, a meta-analysis of 1,441 extensively phenotyped subjects with a broad range of asthma severity from five asthma cohorts was performed. These studies differ in the proportion of subjects with severe asthma. However, these studies used similar comprehensive approaches to characterize subjects with asthma, thus their combined use in a meta-analysis is an appropriate approach. Genomic inflation was weak (inflation factor < 1.1 for every phenotype/population tested) and was adjusted in every population before meta-analysis.

HHIP was the most consistently replicated gene in the five asthmatic populations. HHIP protein is a regulatory factor of the hedgehog signaling pathway interacting directly with all three hedgehog (HH) family members, sonic hedgehog (SHH), indian hedgehog (IHH), and desert hedgehog (DHH).¹⁸ HHIP protein attenuates HH signaling through a negative feedback mechanism by interacting with HH proteins.¹⁸ Patched (PTC) protein prevents HH signaling thorough binding to smoothened (SMO) in the absence of HH; However, HH protein together with cholesterol releases PTC and transduces signals through SMO.^{19,20} In mouse studies, HHIP and Patched homolog 1 (PTCH1) proteins had been shown with a partially overlapping feedback function for HH signaling pathway.²¹ HH protein regulates branching morphogenesis of tracheal-bronchial mesenchyme²² and influences embryonic lung morphogenesis.²³ *HHIP* and *PTCH1* are significantly associated with human height.^{24,25} However, in studies involving measures of lung function such as ppFEV₁ and ppFVC, adjustments for height are used in the calculation of percent predicted values. In GWASs of lung functions primarily in subjects without pulmonary diseases, *HHIP* was associated with FEV₁ and FEV₁/FVC.^{6,7,26} In a GWAS of chronic obstructive pulmonary disease (COPD), *HHIP* was associated with the risk of developing COPD;²⁷ however, since the definition of COPD includes an abnormal level of lung function in a long term smoker, this association may primarily reflect the association with pulmonary function.

The SNPs in *HHIP* most frequently identified in the previous GWAS in non-Hispanic Whites were all in strong LD, and, thus, causal SNPs could not be determined. The SNPs identified previously were either the same or in very strong LD ($R^2 > 0.9$) with the SNPs found in this study.^{6,7} Very importantly, the differences in LD structure between African Americans and non-Hispanic whites allowed identification of a single SNP, rs1512288 (Table I and Fig 1). In our study, the association of *HHIP* with lung functions in non-Hispanic white populations was consistent with the previous GWAS, including SNPs in a large range from 5' flanking region to introns. In African American populations, the association was primarily due to rs1512288, indicating it was either the causal or in LD with

causal SNPs, however only functional studies can determine the true causal SNPs. The effect directions of rs1512288 were the same for non-Hispanic whites and African Americans, i.e., ppFEV₁ increased with copy numbers of minor allele T. The effect size of rs1512288 was larger in African Americans than non-Hispanic whites (Fig 2). Our results confirmed the importance of cross-validation using more than one population with different LD structures. Second-generation sequencing to identify rare SNPs and causal SNPs of *HHIP* in larger African American populations, such as SARP, is warranted in the future.

The SNPs in *HHIP* were also associated with reversibility but not with bronchial hyperresponsiveness (logPC20) (see Table E4 in the Online Repository). Bronchodilator reversibility is a measure of the ability of the lung to return or improve to more nearly normal function. Hence, the association of *HHIP* with bronchodilator reversibility is consistent with the association with baseline lung function. Furthermore, SNPs in *HHIP* were not identified by several other GWASs of asthma susceptibility²⁸⁻³¹ and was not associated with asthma susceptibility in TENOR (see Table E9 in the Online Repository). Based on these data, variation in *HHIP* is important in determining level of lung function in subjects with asthma, which represents an important determinant of asthma severity.

Five genes (*FAM13A*, *NOTCH4-AGER-PPT2*, *THSD4*, *PTCHI*, and *PID1*) were also significantly associated with lung function at the SNP or gene level (Table II and see Table E5 and Table E6 in the Online Repository). *FAM13A* was the only gene (except *HHIP*) replicated at SNP levels, although in FVC instead of FEV₁/FVC as in previous findings.⁶ Interestingly, a recent GWAS of COPD susceptibility identified variants in *FAM13A* (rs7671167 and rs1903003: both in moderate LD ($R^2 > 0.5$) with rs2869967).³¹ Both *HHIP* and *FAM13A* were associated with level of lung function in subjects with and without asthma^{6, 7} and associated with COPD susceptibility,^{27,32} but not asthma susceptibility in previous GWAS, which may suggest genetic differences between the development of asthma or COPD. However, it is important to remember that the definition of COPD is based on abnormal levels of lung function while subjects with asthma may have normal or abnormal levels of baseline lung function, especially those subjects with mild asthma. On the other hand, asthma, COPD, and other lung diseases may share some common genetic pathways. For example, *MMP12* and *ADAM33* have been shown to be associated with lung function, COPD, and asthma.³³⁻³⁵ Genes including *HHIP* and *FAM13A* are associated with normal lung function,^{6,7} COPD susceptibility,^{27,32} and asthma severity; therefore, further study of these candidates with lung function in COPD populations is warranted.³⁵ Normally, a single SNP/gene has limited predictive power for common diseases, and thus, joint analysis of the most consistent SNPs in *HHIP*, *PTCHI*, and *FAM13A* was performed. With the increase in the number of risk SNPs, ppFEV₁, ppFVC, and FEV₁/FVC decreased significantly and consistently for SARP non-Hispanic whites, SARP African Americans and TENOR, and may be generalized to other populations (Table III). Joint analysis of SNPs from 5 replicated genes (*HHIP*, *PTCHI*, *FAM13A*, *PID1*, and *NOTCH4*) generated a more significant trend in SARP non-Hispanic whites, a cohort that was recruited for a wide range of asthma severity (Fig 3A and see Table E8 in the Online Repository). Asthma severity is strongly related to levels of lung function,^{3,4} and there was a significant relationship between the number of risk SNPs and the percent of severe asthmatics based on either ATS criteria or cluster analysis (Fig 3B). Although model over-fitting might be a problem, the results should be generalizable since the SNPs used in the joint analysis were common for normal lung function, COPD susceptibility, and asthma severity and the effect directions were consistent for SARP, TENOR, and CSGA populations.

In summary, these results demonstrate important associations between variation in *HHIP* with lung function in five cohorts of well characterized asthma subjects (n = 1,441). Because of LD among *HHIP* SNPs in white populations, multiple SNPs were significant. However,

in the two African-American cohorts, we narrowed the signal to one SNP (rs1512288). Pulmonary function is a major determinant of asthma severity.¹⁻⁴ Combinations of risk lung function SNPs identified asthmatics with increasing reductions in lung function (Table III and Fig 3). Thus, lung function in these asthma cohorts tracked with the number of risk genotypes and this additive genetic approach could be useful in predicting lung function decline and asthma severity. However, longitudinal studies evaluating changes in asthma lung function in relationship to these genes are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

CSGA	Collaborative Studies on the Genetics of Asthma
FAM13A	family with sequence similarity 13, member A
HHIP	Hedgehog interacting protein
PTCH1	patched homolog 1
ppFEV₁	percentage predicted FEV ₁
SARP	Severe Asthma Research Program
TENOR	The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens

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Figure 1.

Linkage disequilibrium (LD) and association plot of 34 SNPs in *HHIP*. (A) Association plot: blue diamond, red square, green triangle, purple cross, and cyan asterisk represents TENOR, SARP non-Hispanic White, CSGA non-Hispanic White, SARP African American, and CSGA African American population, respectively. (B) LD plot of non-Hispanic Whites. (C) LD plot of African Americans: r^2 color scheme was used and labeled. 95% confidence intervals on D' was used to set up blocks.

Figure 2.

Forest plot of rs1512288 of *HHIP* with *ppFEV*₁. Random effects model are applied on effect size of regression slope. TENOR, SARP_C, CSGA_C, SARP_A, and CSGA_A represents TENOR, SARP non-Hispanic White, CSGA non-Hispanic White, SARP African American, and CSGA African American population, respectively.

Figure 3. Joint analysis of 5 SNPs in *HHIP*, *PTCH1*, *FAM13A*, *PDI1*, and *NOTCH4* in SARP non-Hispanic whites for A: percent predicted FEV₁ and B: percent of subjects with severe asthma based on ATS and cluster classification.^{3,4}

Table I

Meta-analysis results of 34 SNPs in *HHIP* region in five asthmatic populations.

No.	SNP	Minor (risk) Allele C/A [§]	Coordinate	Location	ppFEV ₁			ppFVC			FEV ₁ /FVC		
					P _{meta} [*]	P _{meta_C} [†]	P _{meta_A} [‡]	P _{meta} [*]	P _{meta_C} [†]	P _{meta_A} [‡]	P _{meta} [*]	P _{meta_C} [†]	P _{meta_A} [‡]
1	rs4362772	C	145549252										
2	rs749316	G	145590796		0.029	7.3E-03	0.752	0.046	0.047	0.597	0.734	0.437	0.478
3	rs7684769	T	145638960										
4	rs12641251	G/A	145644293										
5	rs6827794	T	145649747										
6	rs1512282	C	145650947										
7**	rs6845536	C (C)	145672233		2.8E-04	1.1E-04	0.600	2.5E-04	3.2E-04	0.288	0.109	0.077	0.911
8**	rs720485	C (A)	145682038		4.4E-04	2.6E-03	0.067	1.1E-04	1.2E-03	0.035	0.141	0.201	0.462
9**	rs1828591	G (A)	145700230		1.5E-04	1.6E-03	0.036	8.6E-05	1.2E-03	0.023	0.175	0.140	0.901
10***	rs1512288	T (C)	145710731	5'UTR	9.6E-05	9.5E-03	7.7E-04	3.2E-05	4.5E-03	5.1E-04	0.112	0.214	0.300
11	rs2175586	A	145716391										
12	rs1996020	C	145730644										
13	rs7670758	A	145731325		0.010	1.8E-04	0.149	6.9E-03	1.8E-04	0.224	0.242	0.090	0.510
14	rs7677662	G	145732503										
15	rs2353397	C	145737028		0.022	5.9E-03	0.820	0.044	0.012	0.701	0.086	0.089	0.640
16	rs4835180	G	145740058										
17	rs2035901	G/A	145741317		0.340	0.232	0.854	0.054	0.033	0.920	0.721	0.485	0.593
18	rs11947381	T	145757300		0.851	0.651	0.232	0.022	1.8E-03	0.356	0.097	0.100	0.651
19	rs2575570	T	145768383										
20	rs1398244	T	145781907		0.258	0.464	0.314	0.734	0.600	0.804	0.005	0.007	0.355
21	rs6845999	T	145785276										
22	rs1812175	T	145794294										
23	rs2220514	T	145796008	intron									
24	rs2306924	A	145801986										
25	rs6537307	G	145821313										

No.	SNP	Minor (risk) Allele C/A [§]	Coordinate	Location	ppFEV ₁			ppFVC			FEV ₁ /FVC		
					P _{meta} [*]	P _{meta_C} [‡]	P _{meta_A} [‡]	P _{meta} [*]	P _{meta_C} [‡]	P _{meta_A} [‡]	P _{meta} [*]	P _{meta_C} [‡]	P _{meta_A} [‡]
26	rs2575580	C	145824475		0.936	0.600	0.270	0.270	0.929	0.033	0.099	0.132	0.496
27	rs6537310	T	145856182		0.275	0.108	0.523	0.074	7.3E-03	0.240	0.996	0.934	0.872
28	rs6854783	G	145862529		0.264	0.107	0.552	0.055	7.3E-03	0.373	0.960	0.895	0.729
29	rs11944404	C	145890321		0.034	0.142	0.088	0.297	0.535	0.300	0.133	0.211	0.396
30	rs4835186	T	145906597										
31	rs2353934	T	145916490	3'UTR	0.446	0.911	0.173	0.125	0.516	0.045	0.928	0.876	0.922
32	rs12647866	C	145928136										
33	rs12651029	A	145989617		0.040	0.020	0.963	0.013	3.2E-03	0.822	0.852	0.651	0.662
34	rs13102609	T	145990671										

Only rows with at least one entry with P value less than 0.05 are showed.

^{*} P_{meta} is the meta-analysis P value for all five asthmatic populations.

[‡] P_{meta_C} is the meta-analysis P value for three non-Hispanic White populations.

[‡] P_{meta_A} is the meta-analysis P value for two African American populations.

[§] Minor (risk) Allele C/A represents minor (risk) allele of non-Hispanic White and African American populations, respectively. Risk alleles are labeled for SNPs 7-10 only. Please refer to Table E2 and E3 for other risk alleles.

^{**} SNPs 7-10 are the four SNPs studied for strict replication since they are in strong LD with the previously identified SNP. Results from the other previously genotyped SNPs are presented for completeness but are the results of a secondary analysis.

Table II

Meta-analysis results of 10 candidate genes in five asthmatic populations.

Previously Identified SNPs	Tested SNPs	LD C/A§	Minor (risk) Allele C/A§	ppFEV ₁				ppFVC				FEV ₁ /FVC			
				P _{meta} *	P _{meta} C [†]	P _{meta} A [‡]	P _{meta} *	P _{meta} C [†]	P _{meta} A [‡]	P _{meta} *	P _{meta} C [†]	P _{meta} A [‡]	P _{meta} *	P _{meta} C [†]	P _{meta} A [‡]
<i>TNSI</i> : rs2571445	rs2571445	1	T	0.14	0.47	0.08	0.11	0.47	0.05	0.71	0.76	0.83			
	rs929937	0/0	C/T	0.32	0.64	0.22	0.01	0.06	0.02	0.56	0.43	0.80			
<i>INTS12-GSTCD-NPNT</i> : rs10516526	rs10516526	1	G	0.80	0.96	0.68	0.61	0.91	0.21	0.68	0.79	0.70			
	rs3960769	0/0	A	0.08	0.12	0.41	0.47	0.81	0.28	0.01	0.06	0.05			
<i>HTR4</i> : rs3995090	rs3995090	1	C	0.10	0.14	0.45	0.43	0.37	1.00	0.24	0.51	0.22			
	rs1833710	0/0	C	0.04	0.01	0.63	0.02	0.01	0.97	0.67	0.24	0.20			
<i>NOTCH4-AGER-PPT2</i> : rs2070600	rs2070600	1	A	0.33	0.91	0.07	0.74	0.43	0.45	0.36	0.79	0.02			
	rs206015	0.7/NA	T	3.2E-03	2.0E-04	0.51	0.01	9.0E-04	0.63	0.47	0.22	0.47			
<i>THSD4</i> : rs12899618	rs1913768	1/0.8	A	0.32	0.41	0.58	0.69	0.96	0.36	0.04	0.07	0.41			
	rs1568010	0.2/0	C	0.09	0.12	0.50	0.82	0.83	0.38	2.9E-03	0.05	0.01			
<i>GPR126</i> : rs11155242	rs11155242	1	C	0.16	0.16	0.70	0.95	0.75	0.65	0.25	0.22	0.87			
	rs171891	0.9/0.6	A	0.02	0.02	0.59	0.91	0.95	0.91	0.36	0.37	0.81			
<i>ADAM19</i> : rs2277027	rs2277027	1	C/A	0.59	0.46	0.83	0.54	0.82	0.40	0.98	0.64	0.36			
	rs6890282	0/0	G/T	0.03	0.18	0.05	0.69	0.71	0.88	0.16	0.57	0.06			
<i>FAM13A</i> : rs2869967	rs2869967	1	C/T (C/T)	0.26	0.17	0.88	2.9E-03	4.9E-03	0.29	0.20	0.09	0.69			
	rs6830970	0.6/0.2	G	0.10	0.15	0.44	1.6E-03	0.01	0.09	0.36	0.12	0.34			
<i>PTCH1</i> : rs16909898	rs10512249	1/0.6	T	0.72	0.93	0.57	0.22	0.24	0.69	0.51	0.76	0.41			
	rs576594	0.1/0.2	T (T/C)	9.0E-04	6.5E-07	0.04	0.01	1.9E-05	0.03	0.01	1.1E-03	0.90			
<i>PID1</i> : rs1435867	rs1358443	0.3/0.1	C	0.18	0.44	0.16	0.31	0.20	0.84	0.04	0.36	0.01			
	rs3845823	0/0	T	0.02	0.01	0.81	3.8E-04	1.0E-03	0.17	0.86	0.56	0.48			

The first SNP in each gene was tested under our primary hypothesis of testing the previously reported SNP (or one in strong LD) for strict replication of the results in the general population. The second SNP listed for each gene is the SNP with the strongest p-value across the gene to present a more comprehensive view of each gene (secondary analysis).

- * P_{meta} is the meta-analysis P value for all five asthmatic populations.
- [†] $P_{\text{meta\_C}}$ is the meta-analysis P value for three non-Hispanic White populations.
- [‡] $P_{\text{meta\_A}}$ is the meta-analysis P value for two African American populations.
- [§] LD C/A and Minor (risk) Allele C/A represent R^2 of LD between tested SNPs and candidate SNPs and minor (risk) allele of non-Hispanic White and African American populations, 517 respectively. Risk alleles are labeled for rs2869967 and rs576594 only. Please refer to Table E5 and E6 for other risk alleles.

Table III

Joint analysis of 3 SNPs in *HHIP*, *PTCH1*, and *FAM13A*.

SARP non-Hispanic whites					
Number of risk SNPs *	0	1	2	3	P value R ²
N	42	141	175	71	
ppFEV ₁	83 ± 22	80 ± 21	74 ± 23	73 ± 22	0.003 0.02
ppFVC	92 ± 19	90 ± 17	85 ± 20	84 ± 17	0.004 0.02
FEV ₁ /FVC	0.75 ± 0.1	0.72 ± 0.1	0.71 ± 0.1	0.70 ± 0.1	0.03 0.01
TENOR non-Hispanic whites					
Number of risk SNPs *	0	1	2	3	P value R ²
N	47	137	192	60	
ppFEV ₁	87 ± 26	78 ± 19	78 ± 20	72 ± 23	0.003 0.02
ppFVC	91 ± 21	89 ± 17	90 ± 17	84 ± 19	0.08 0.007
FEV ₁ /FVC	0.77 ± 0.1	0.72 ± 0.1	0.72 ± 0.1	0.71 ± 0.1	0.007 0.02
SARP African Americans					
Number of risk SNPs *	0	1	2	3	P value R ²
N	15	78	82	30	
ppFEV ₁	85 ± 26	80 ± 20	75 ± 20	71 ± 17	0.005 0.04
ppFVC	97 ± 23	91 ± 19	88 ± 17	88 ± 16	0.06 0.02
FEV ₁ /FVC	0.72 ± 0.1	0.74 ± 0.1	0.71 ± 0.1	0.69 ± 0.1	0.07 0.02

* Number of risk SNPs is the sum of risk SNPs in *HHIP* (rs1512288), *PTCH1* (rs576594), and *FAM13A* (rs2869967).