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

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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 white and African American subjects with asthma is unknown.

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

Methods: Single nucleotide polymorphisms (SNPs) were tested in 5 asthma populations (N = 1441) 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. 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 5 populations (rs1512288: P_{meta} = 9.62E-05 and 3.23E-05 for percent predicted FEV_1 [ppFEV_1] and percent predicted forced vital capacity [ppFVC], respectively). The SNPs in *HHIP* were also associated with reversibility ($P < .05$) but not bronchial hyperresponsiveness to methacholine. 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 white and African American subjects with asthma.

Conclusion: A subset of the genes, including *HHIP*, that regulate lung function in general populations are associated with abnormal lung function in asthma in non-Hispanic white and African American subjects. (J Allergy Clin Immunol 2011;127:1457-65.)

Key words: *Asthma, genetics, asthma severity, meta-analysis, FEV_1, FVC, FEV_1/FVC, HHIP, FAM13A, PTCH1*

Asthma is a heterogeneous disease that is classified phenotypically as mild, moderate, or severe on the basis of National Asthma Education and Prevention Program (NAEPP), Global Initiative for Asthma (GINA), or American Thoracic Society (ATS) guidelines,[1-3] and more recently, 5 asthma severity

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Abbreviations used
ATS: American Thoracic Society
COPD: Chronic obstructive pulmonary disease
CSGA: Collaborative Studies on the Genetics of Asthma
FAM13A: Family with sequence similarity 13, member A
FVC: Forced vital capacity
GWAS: Genome-wide association study
HHIP: Hedgehog interacting protein
LD: Linkage disequilibrium
PTCH1: Patched homolog 1
pp: Percent predicted
SARP: Severe Asthma Research Program
SNP: Single nucleotide polymorphism
TENOR: The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens

phenotypes were identified by using cluster analysis.[4] The majority of morbidity and health care use occurs in severe asthma, a phenotype largely determined by pulmonary function (baseline lung function: percent predicted [pp] FEV_1, pp forced vital capacity [FVC], and FEV_1/FVC).[3,4] Quantitative phenotypes such as $ppFEV_1$ are essential for categorizing asthma severity for current guidelines classification or with cluster methodologies.[5]

A recent genome-wide association study (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 FEV_1, and 8 genes (hedgehog interacting protein [*HHIP*], *GPR126*, *ADAM19*, *AGER-PPT2*, family with sequence similarity 13, member A [*FAM13A*], patched homolog 1 [*PTCH1*], *PID1*, and *HTR4*) were associated with FEV_1/FVC.[6] A second GWAS meta-analysis for lung function in general populations (20,288 participants of European white ancestry: SpiroMeta consortium) identified 4 genes (*HHIP*, *GSTCD*, *TNS1*, and *HTR4*) associated with FEV_1 and 3 loci (*HHIP*, *NOTCH4-AGER-PPT2*, *THSD4*) associated with FEV_1/FVC.[7] Although 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 $ppFEV_1$, ppFVC, and FEV_1/FVC in 5 asthma populations including non-Hispanic whites and African Americans to determine whether the genes previously identified in the 2 GWASs 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 single nucleotide polymorphism (SNP) in each gene that was significant in the general population is important in asthma; therefore, we analyzed 1 SNP in each of the 11 genes (3 additional SNPs were analyzed in *HHIP* because they are in strong linkage disequilibrium (LD) with the previously identified SNP). 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 National Heart, Lung, and Blood Institute–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) multicenter study.[11,12] In addition, subjects with asthma who were studied in the National Heart, Lung, and Blood Institute 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 by using standard protocols, and SNP genotyping was performed by using the Illumina HumanCNV370 BeadChip (Illumina, Inc, San Diego, Calif) for TENOR.[12] SARP and CSGA samples were genotyped using the Illumina HumanHap1M BeadChip (unpublished data). Genotyping for both studies was performed by 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 ≥0.9), (3) were different from described race, (4) failed the check for family relatedness (PI_HAT > 0.125), or (5) were detected as an outlier (>6 SDs 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 ($P < 10E-04$), or (3) the minor allele frequency 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 with PLINK (version 1.06; http://pngu.mgh.harvard.edu/purcell/plink/),[13] adjusted for age, sex, and significant principal components (EIGENSTRAT, version 3.0; http://genepath.med.harvard.edu/~reich/Software.htm).[14] Haploview (http://www.broad.mit.edu/mpg/haploview/) was used to generate linkage disequilibrium plots,[15] and 95% CIs on D' were used to define blocks.[16] For the primary analysis, 14 SNPs in 11 candidate genes/regions were analyzed for replication at the SNP level (rs6845536, rs720485, rs1828591, and rs1512288 in *HHIP*; rs2571445 in *TNS1*; 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 3 non-Hispanic white populations, 2 African American populations, or all of 5 populations was performed with weights proportional to the square-root of the sample size by using METAL software (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 the rmeta package based on the random effects model (http://cran.r-project.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 the 3 most consistent SNPs (rs1512288 in *HHIP*, rs576594 in *PTCH1*, and rs2869967 in *FAM13A*) or 5 SNPs (rs1512288 in *HHIP*, rs576594 in *PTCH1*, rs2869967 in *FAM13A*, rs3845823 in *PID1*, and rs206015 in *NOTCH4*) was performed in SARP non-Hispanic white subjects, SARP African American subjects, and TENOR white subjects. 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 $ppFEV_1$, ppFVC, and FEV_1/FVC with the number of risk SNPs. A logistic regression model was used for analysis of the percentage of subjects with severe asthma by using ATS and cluster classification[3,4] with the number of risk SNPs.

TABLE I. Meta-analysis results of 34 SNPs in the *HHIP* region in 5 populations with asthma

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	5′UTR									
2	rs749316	G	145590796		.029	7.3E-03	.752	.046	.047	.597	.734	.437	.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	.600	2.5E-04	3.2E-04	.288	.109	.077	.911
8‖	rs720485	C (A)	145682038		4.4E-04	2.6E-03	.067	1.1E-04	1.2E-03	.035	.141	.201	.462
9‖	rs1828591	G (A)	145700230		1.5E-04	1.6E-03	.036	8.6E-05	1.2E-03	.023	.175	.140	.901
10‖	rs1512288	T (C)	145710731		9.6E-05	9.5E-03	7.7E-04	3.2E-05	4.5E-03	5.1E-04	.112	.214	.300
11	rs2175586	A	145716391										
12	rs1996020	C	145730644										
13	rs7670758	A	145731325		.010	1.8E-04	.149	6.9E-03	1.8E-04	.224	.242	.090	.510
14	rs7677662	G	145732503										
15	rs2353397	C	145737028		.022	5.9E-03	.820	.044	.012	.701	.086	.089	.640
16	rs4835180	G	145740058										
17	rs2035901	G/A	145741317		.340	.232	.854	.054	.033	.920	.721	.485	.593
18	rs11947381	T	145757300		.851	.651	.232	.022	1.8E-03	.356	.097	.100	.651
19	rs2575570	T	145768383										
20	rs1398244	T	145781907		.258	.464	.314	.734	.600	.804	.005	.007	.355
21	rs6845999	T	145785276										
22	rs1812175	T	145794294	Intron									
23	rs2220514	T	145796008										
24	rs2306924	A	145801986										
25	rs6537307	G	145821313										
26	rs2575580	C	145824475		.936	.600	.270	.270	.929	.033	.099	.132	.496
27	rs6537310	T	145856182		.275	.108	.523	.074	7.3E-03	.240	.996	.934	.872
28	rs6854783	G	145862529		.264	.107	.552	.055	7.3E-03	.373	.960	.895	.729
29	rs11944404	C	145890321	3′UTR	.034	.142	.088	.297	.535	.300	.133	.211	.396
30	rs4835186	T	145906597										
31	rs2353934	T	145916490		.446	.911	.173	.125	.516	.045	.928	.876	.922
32	rs12647866	C	145928136										
33	rs12651029	A	145989617		.040	.020	.963	.013	3.2E-03	.822	.852	.651	.662
34	rs13102609	T	145990671										

UTR, Untranslated region.

Only rows with at least 1 entry with a *P* value less than .05 are shown.

*P_{meta} is the meta-analysis *P* value for all 5 populations with asthma.

†P_{meta_C} is the meta-analysis *P* value for 3 non-Hispanic white populations.

‡P_{meta_A} is the meta-analysis *P* value for 2 African American populations.

§Minor (risk) allele C/A represents the minor (risk) allele of non-Hispanic white and African American populations, respectively. Risk alleles are labeled for SNPs 7 to 10 only. See Tables E2 and E3 for other risk alleles.

‖SNPs 7 to 10 are the 4 SNPs studied for strict replication because 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.

RESULTS

Although the 3 studies used similar clinical approaches to phenotype cases carefully, they differ in the proportion of subjects with severe asthma because SARP is a cohort that is enriched for subjects with severe asthma, TENOR focused on difficult-to-treat or severe asthma, and 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 this article's Table E1 in the Online Repository at www.jacionline.org). 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, data from 1441 subjects with asthma (SARP non-Hispanic white subjects, n = 438; TENOR non-Hispanic white subjects, n = 431; CSGA non-Hispanic white subjects, n = 230; SARP African American subjects, n = 206; and CSGA African American subjects, n = 136) were analyzed for association with spirometric measures of lung function: ppFEV$_1$, ppFVC, and FEV$_1$/FVC, and the SNPs in 11 genes identified through 2 recent meta-analyses of GWAS of normal lung function. The issue of multiple testing was minimized because only 14 SNPs in 11 previously identified genes were analyzed for replication at the SNP level (*P* value for Bonferroni adjustment was 3.57E-03; *P* = .05/14 SNPs).

The SNP rs1512288 at the 5' flanking region of *HHIP* showed the most consistent association in all 5 cohorts of subjects with asthma (P_{meta} = 9.62E-05, 3.23E-05, and .11 for ppFEV$_1$, ppFVC, and FEV$_1$/FVC, respectively; Table I; Fig 1, *A*; see this article's Tables E2 and E3 in the Online Repository at www.jacionline.org). The LD structure of the *HHIP* region was different between non-Hispanic white and African American subjects (Fig 1, *B* and *C*). The SNPs with the lowest *P* values

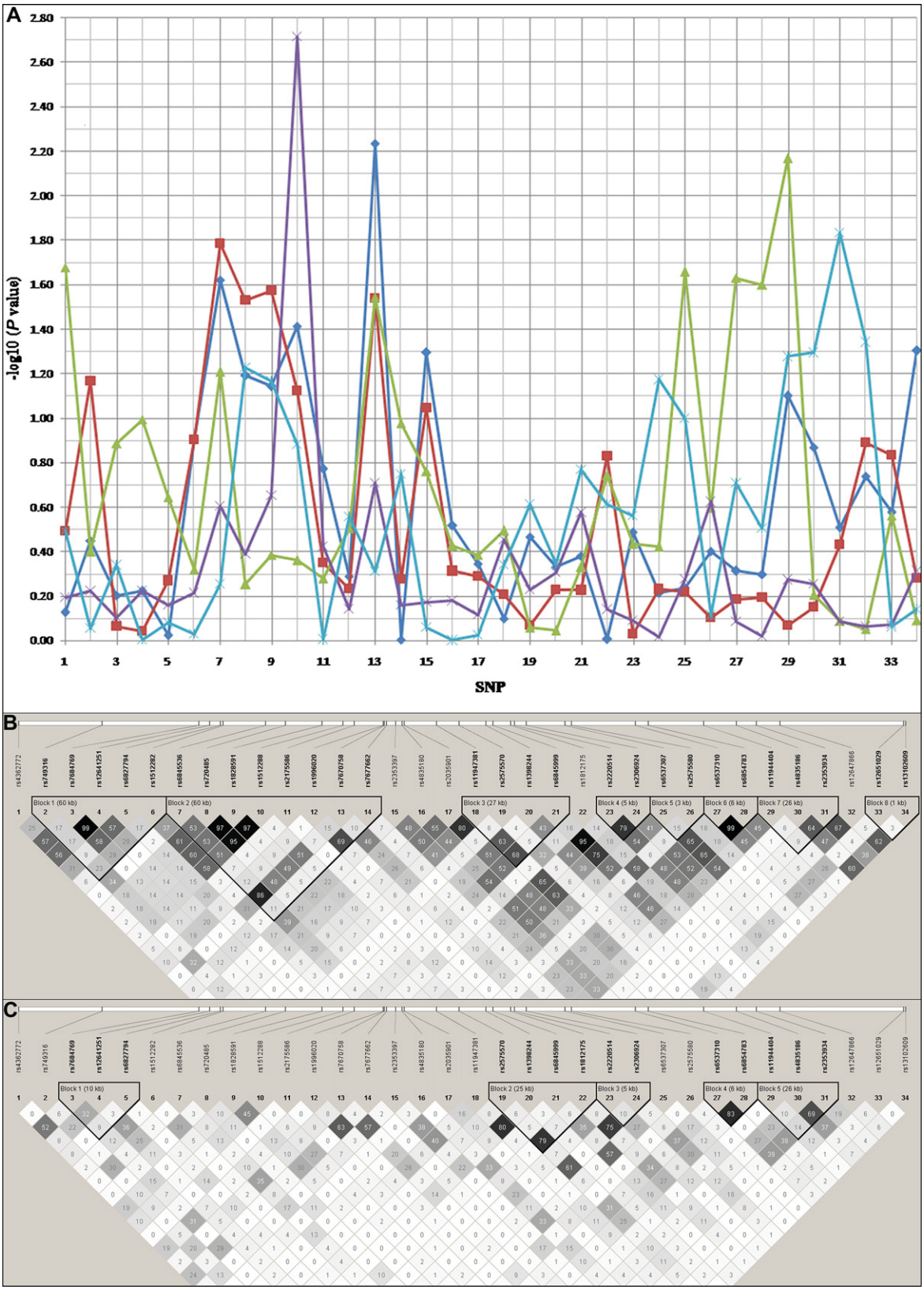

FIG 1. LD and association plot of 34 SNPs in *HHIP*. **A,** Association plot: *blue diamond,* TENOR subjects; *red square,* SARP non-Hispanic white subjects; *green triangle,* CSGA non-Hispanic white subjects; *purple cross,* SARP African American subjects; *cyan asterisk,* CSGA African American subjects. **B,** LD plot of non-Hispanic white subjects. **C,** LD plot of African American subjects: r^2 color scheme was used and labeled. 95% CIs on D′ were used to set up blocks.

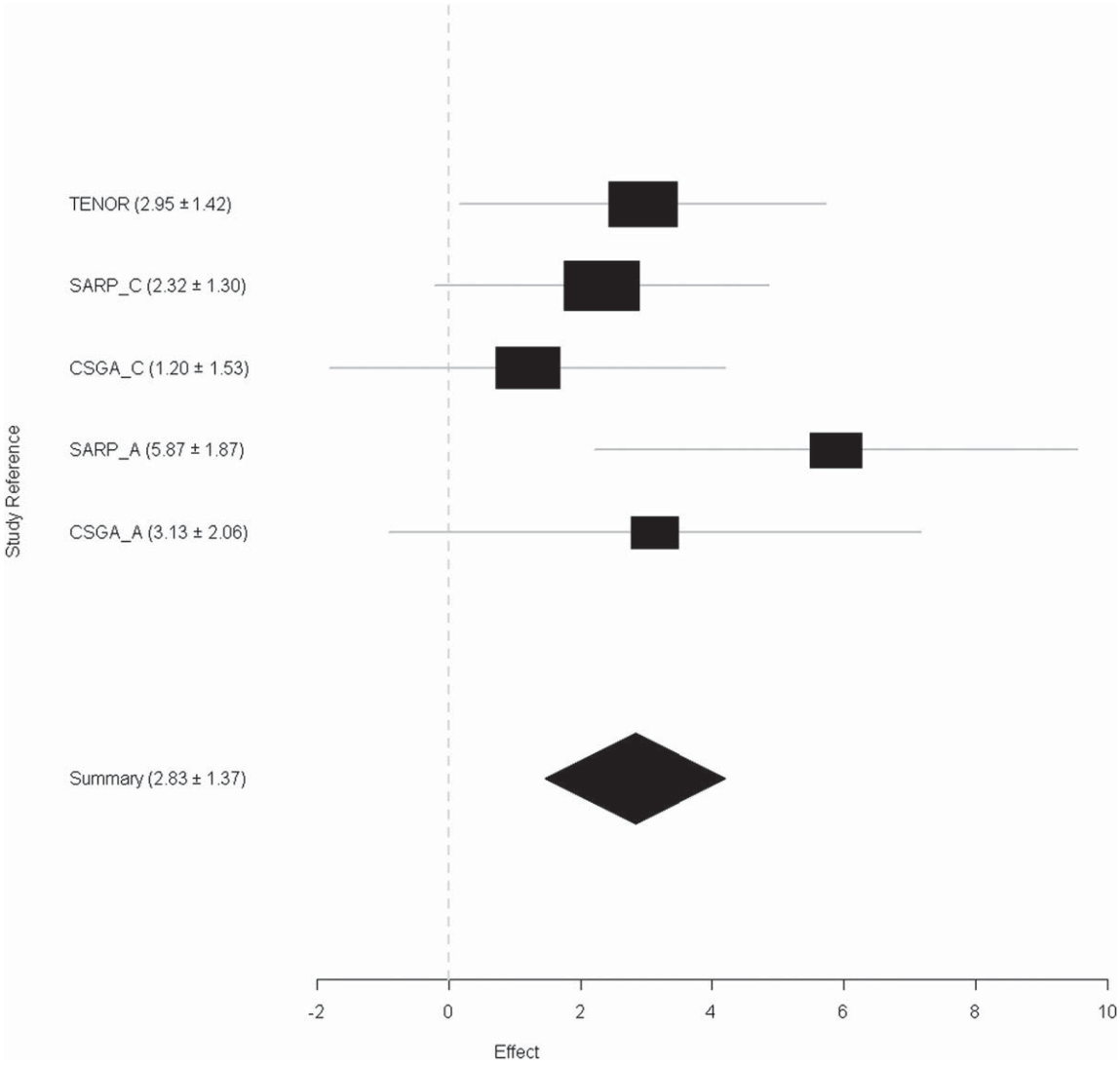

FIG 2. Forest plot of rs1512288 of *HHIP* with ppFEV$_1$. Random effects model are applied on effect size of regression slope. *SARP_C,* SARP non-Hispanic white subjects; *CSGA_C,* CSGA non-Hispanic white subjects; *SARP_A,* SARP African American subjects; *CSGA_A,* CSGA African American subjects.

—rs6845536, rs720485, rs1828591, and rs1512288—were in strong LD in non-Hispanic white subjects ($R^2 > 0.5$), but in weak LD in African American subjects ($R^2 < 0.5$). SNP rs6845536 at the 5' flanking region of *HHIP*, in LD with rs1512288 ($R^2 = 0.63$), showed the strongest signal in non-Hispanic white populations (P_{meta} = 1.08E-04, 3.20E-04, and .08 for ppFEV$_1$, ppFVC, and FEV$_1$/FVC, respectively), whereas SNP rs1512288 had the strongest signal in African American subjects (P_{meta} = 7.74E-04, 5.11E-04, and .30 for ppFEV$_1$, ppFVC, and FEV$_1$/FVC, respectively). Thus, we conclude on the basis of the LD structure in African American subjects that rs1512288 is either the causal SNP or in LD with a smaller set of SNPs (Table I; Fig 1).

The effect size (regression slope) of rs1512288 was consistent among 5 asthma populations (heterogeneity *P* value = .41; Fig 2). The summary effect size was 2.83 (SD = 1.37), and ppFEV$_1$ increased 2.83% on average with every copy of the minor allele T. For non-Hispanic white subjects from the SARP cohort, the effect size was 2.32 (SD = 1.30) for ppFEV$_1$ or 0.085 (SD = 0.056) for baseline FEV$_1$, and FEV$_1$ differed by 85 mL on average with every copy of the minor allele T; for African American subjects from SARP, the effect size was 5.87 (SD = 1.87) for ppFEV$_1$ or 0.252 (SD = 0.077) for baseline FEV$_1$, and FEV$_1$ differed by 252 mL on average with every copy of the minor allele T.

Single nucleotide polymorphisms in *HHIP* were associated with bronchodilator reversibility (rs720485: *P* = .024 and .057 for non-Hispanic white and African American subjects, respectively) but not with bronchial hyperresponsiveness to methacholine (logPC$_{20}$; see this article's Table E4 in the Online Repository at www.jacionline.org).

Analysis results from the other genes previously identified for lung function in general populations identified 5 genes (*FAM13A*, *NOTCH4*, *THSD4*, *PTCH1*, and *PID1*) that were associated significantly with lung function in asthma at the SNP or gene level (Table II; see this article's Tables E5 and E6 in the Online Repository at www.jacionline.org). At the SNP level, rs2869967, in the intron of *FAM13A* on chromosome 4q22, was significant (P_{meta} = 2.9E-03 for FVC) using strict replication standards and after

TABLE II. Meta-analysis results of 10 candidate genes in 5 populations with asthma

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}‡
TNS1: rs2571445	rs2571445	1	T	.14	.47	.08	.11	.47	.05	.71	.76	.83
	rs929937	0/0	C/T	.32	.64	.22	.01	.06	.02	.56	.43	.80
INTS12-GSTCD-NPNT: rs10516526	rs10516526	1	G	.80	.96	.68	.61	.91	.21	.68	.79	.70
	rs3960769	0/0	A	.08	.12	.41	.47	.81	.28	.01	.06	.05
HTR4: rs3995090	rs3995090	1	C	.10	.14	.45	.43	.37	1.00	.24	.51	.22
	rs1833710	0/0	C	.04	.01	.63	.02	.01	.97	.67	.24	.20
NOTCH4-AGER-PPT2: rs2070600	rs2070600	1	A	.33	.91	.07	.74	.43	.45	.36	.79	.02
	rs206015	0.7/NA	T	3.2E-03	2.0E-04	.51	.01	9.0E-04	.63	.47	.22	.47
THSD4: rs12899618	rs1913768	1/0.8	A	.32	.41	.58	.69	.96	.36	.04	.07	.41
	rs1568010	0.2/0	C	.09	.12	.50	.82	.83	.38	2.9E-03	.05	.01
GPR126: rs11155242	rs11155242	1	C	.16	.16	.70	.95	.75	.65	.25	.22	.87
	rs171891	0.9/0.6	A	.02	.02	.59	.91	.95	.91	.36	.37	.81
ADAM19: rs2277027	rs2277027	1	C/A	.59	.46	.83	.54	.82	.40	.98	.64	.36
	rs6890282	0/0	G/T	.03	.18	.05	.69	.71	.88	.16	.57	.06
FAM13A: rs2869967	rs2869967	1	C/T (C/T)	.26	.17	.88	2.9E-03	4.9E-03	.29	.20	.09	.69
	rs6830970	0.6/0.2	G	.10	.15	.44	1.6E-03	.01	.09	.36	.12	.34
PTCH1: rs16909898	rs10512249	1/0.6	T	.72	.93	.57	.22	.24	.69	.51	.76	.41
	rs576594	0.1/0.2	T (T/C)	9.0E-04	6.5E-07	.04	.01	1.9E-05	.03	.01	1.1E-03	.90
PID1: rs1435867	rs1358443	0.3/0.1	C	.18	.44	.16	.31	.20	.84	.04	.36	.01
	rs3845823	0/0	T	.02	.01	.81	3.8E-04	1.0E-03	.17	.86	.56	.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 5 populations with asthma.
†P_{meta_C} is the meta-analysis *P* value for 3 non-Hispanic white populations.
‡P_{meta_A} is the meta-analysis *P* value for 2 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, respectively. Risk alleles are labeled for rs2869967 and rs576594 only. See Tables E5 and E6 for other risk alleles.

correction for multiple testing. This signal was driven mainly by non-Hispanic white subjects. *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. *TNS1* 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 the hedgehog signaling pathway. SNP rs576594 was associated with ppFEV$_1$ (P_{meta} = 6.5E-07), ppFVC (P_{meta} = 1.9E-05), and FEV$_1$/FVC (P_{meta} = 1.1E-03) in the white populations, specifically the extensively phenotyped SARP cohort with broader ranges of asthma severity and lung function (Tables II, E5, and E6). The association of rs576594 was observed in the African American subjects, although weaker ($P < .05$) and, interestingly, in the opposite direction. Because HHIP and PTCH1 had overlapping functions in the hedgehog signaling pathway, the 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 3 white populations; however, interaction ($P < .05$) was observed in the 2 African American populations (rs3824491: P = 8.5E-03, .071, and .051 for meta-analysis, SARP, and CSGA African American subjects, respectively; see this article's Table E7 in the Online Repository at www.jacionline.org). This interaction needs to be replicated in other populations because the 2 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, ppFEV$_1$, ppFVC, and FEV$_1$/FVC decreased (range of *P* values, .08-.003; Table III). The results were consistent for SARP non-Hispanic white subjects, SARP African American subjects, and TENOR, and may be generalized to other populations. Modeling with the 3 best SNPs, limited variance (0.7% to 4%) was explained (Table III), indicating there may be 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 white subjects, a cohort that was recruited for a wide range of asthma severity and pulmonary function (P = 9.8E-06 for ppFEV$_1$; Fig 3, *A*; see this article's Table E8 in the Online Repository at www.jacionline.org), and less significant in TENOR, a cohort with a narrower range of lung function (P = .01 for ppFEV$_1$). In SARP non-Hispanic white subjects, ppFEV$_1$ 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 3, *A*). Because of this relationship, we analyzed 2 measures of asthma severity in the SARP non-Hispanic white subjects. The first was based on ATS criteria for severe asthma[3] by comparing severe asthma (group 5) and mild asthma (groups 1 and 2), whereas the second used our newer approach to asthma phenotypic classification based on cluster analysis[4] by comparing the more severe clusters (clusters 3, 4, and 5) with the less severe clusters (clusters 1 and 2). As seen in Fig 3, *B,* there is an increase in severity as the number of

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

No. of risk SNPs*	0	1	2	3	*P* value	R^2
SARP non-Hispanic white subjects						
n	42	141	175	71		
$ppFEV_1$	83 ± 22	80 ± 21	74 ± 23	73 ± 22	.003	0.02
ppFVC	92 ± 19	90 ± 17	85 ± 20	84 ± 17	.004	0.02
FEV_1/FVC	0.75 ± 0.1	0.72 ± 0.1	0.71 ± 0.1	0.70 ± 0.1	.03	0.01
TENOR non-Hispanic white subjects						
n	47	137	192	60		
$ppFEV_1$	87 ± 26	78 ± 19	78 ± 20	72 ± 23	.003	0.02
ppFVC	91 ± 21	89 ± 17	90 ± 17	84 ± 19	.08	0.007
FEV_1/FVC	0.77 ± 0.1	0.72 ± 0.1	0.72 ± 0.1	0.71 ± 0.1	.007	0.02
SARP African American subjects						
n	15	78	82	30		
$ppFEV_1$	85 ± 26	80 ± 20	75 ± 20	71 ± 17	.005	0.04
ppFVC	97 ± 23	91 ± 19	88 ± 17	88 ± 16	.06	0.02
FEV_1/FVC	0.72 ± 0.1	0.74 ± 0.1	0.71 ± 0.1	0.69 ± 0.1	.07	0.02

*No. of risk SNPs is the sum of risk SNPs in *HHIP* (rs1512288), *PTCH1* (rs576594), and *FAM13A* (rs2869967).

risk SNPs increases (P = 6.0E-04 and 2.7E-03 for ATS and cluster classification, respectively).

DISCUSSION

In this study, a meta-analysis of 1441 extensively phenotyped subjects with a broad range of asthma severity from 5 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 5 asthmatic populations. HHIP protein is a regulatory factor of the hedgehog signaling pathway, interacting directly with all 3 hedgehog family members: sonic hedgehog, Indian hedgehog, and desert hedgehog.[18] HHIP protein attenuates hedgehog signaling through a negative feedback mechanism by interacting with hedgehog proteins.[18] Patched protein prevents hedgehog signaling through binding to smoothened in the absence of hedgehog; however, hedgehog protein together with cholesterol releases patched and transduces signals through smoothened.[19,20] In mouse studies, HHIP and PTCH1 proteins had been shown with a partially overlapping feedback function for hedgehog signaling pathway.[21] Hedgehog protein regulates branching morphogenesis of tracheal-bronchial mesenchyme[22] 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 $ppFEV_1$ 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 FEV_1 and FEV_1/FVC.[6,7,26] In a GWAS of chronic obstructive pulmonary disease (COPD), *HHIP* was associated with the risk of developing COPD[27]; however, because 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 white subjects 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 American and non-Hispanic white subjects allowed identification of a single SNP, rs1512288 (Table I; 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 the 5' flanking region to introns. In African American populations, the association was primarily a result of rs1512288, indicating it was either the causal SNP 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 white and African American subjects —that is, $ppFEV_1$ increased with copy numbers of minor allele T. The effect size of rs1512288 was larger in African American than non-Hispanic white subjects (Fig 2). Our results confirmed the importance of cross-validation by using more than 1 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 ($logPC_{20}$; Table E4). 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 susceptibility[28-31] and were not associated with asthma susceptibility in TENOR (see this article's Table E9 in the Online Repository at www.jacionline.org). On the basis of 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*, *PTCH1*, and *PID1*) were also significantly associated with lung function at the SNP or gene level (Tables II, E5, and E6). *FAM13A* was the only gene (except *HHIP*) replicated at SNP levels, although in FVC instead of FEV_1/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).[32] Both *HHIP* and

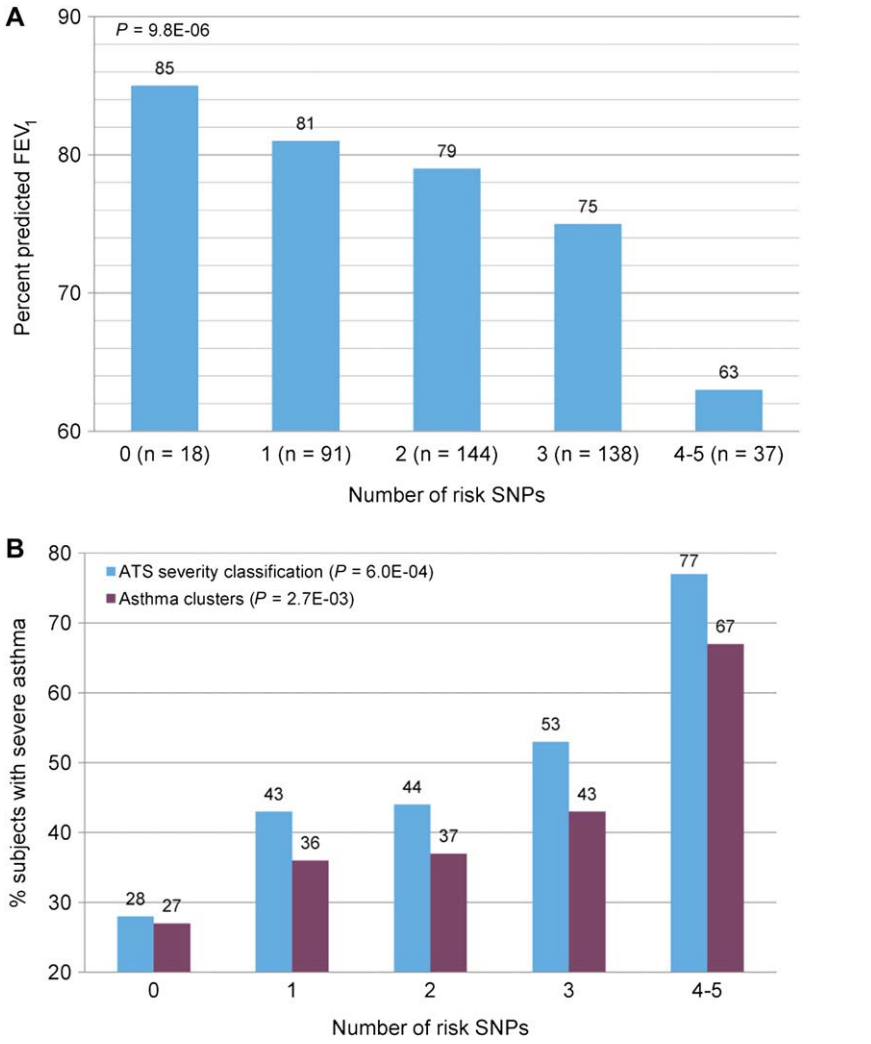

FIG 3. Joint analysis of 5 SNPs in *HHIP, PTCH1, FAM13A, PID1,* and *NOTCH4* in SARP non-Hispanic white subjects for $ppFEV_1$ **(A)** and percentage of subjects with severe asthma **(B)** on the basis of ATS and cluster classification.[3,4]

FAM13A were associated with level of lung function in subjects with and without asthma[6,7] and were associated with COPD susceptibility[27,32] but not asthma susceptibility in previous GWASs, which may suggest genetic differences between the development of asthma and COPD. However, it is important to remember that the definition of COPD is based on abnormal levels of lung function, whereas 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*, *PTCH1*, and *FAM13A* was performed. With the increase in the number of risk SNPs, $ppFEV_1$, ppFVC, and FEV_1/FVC decreased significantly and consistently for SARP non-Hispanic white subjects, SARP African American subjects, and TENOR, and may be generalized to other populations (Table III). Joint analysis of SNPs from 5 replicated genes (*HHIP*, *PTCH1*, *FAM13A*, *PID1*, and *NOTCH4*) generated a more significant trend in SARP non-Hispanic white subjects, a cohort that was recruited for a wide range of asthma severity (Fig 3, *A*; Table E8). 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 percentage of patients with severe asthma on the basis of either ATS criteria or cluster analysis (Fig 3, *B*). Although model overfitting might be a problem, the results should be generalizable because 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 5 cohorts of well characterized subjects with asthma (N = 1441). Because of LD among *HHIP* SNPs in white populations, multiple SNPs were

significant. However, in the 2 African American cohorts, we narrowed the signal to 1 SNP (rs1512288). Pulmonary function is a major determinant of asthma severity.[1-4] Combinations of risk lung function SNPs identified patients with asthma with increasing reductions in lung function (Table III; 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.

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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 patients with asthma with lower levels of lung function.

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