

Dual-specificity phosphatase 1 as a pharmacogenetic modifier of inhaled steroid response among asthmatic patients

Ying Jin, BS,^{a,b,*} Donglei Hu, PhD,^{c,*} Edward L. Peterson, PhD,^{d,*} Celeste Eng, BS,^c Albert M. Levin, PhD,^d Karen Wells, BS,^d Kenneth Beckman, PhD,^e Rajesh Kumar, MD,^f Max A. Seibold, PhD,^g Gloria Karungi, MBChB, MS,^a Amanda Zoratti, BA,^a John Gaggin, BSN,^a Janis Campbell, RN,^a Joshua Galanter, MD,^c Rocío Chapela, MD,^h José R. Rodríguez-Santana, MD,ⁱ H. Geoffrey Watson, MD,^j Kelley Meade, MD,^k Michael LeNoir, MD,^l William Rodríguez-Cintrón, MD,^m Pedro C. Avila, MD,ⁿ David E. Lanfear, MD, MS,^{a,o} Esteban G. Burchard, MD, MPH,^{c,p,*} and L. Keoki Williams, MD, MPH^{d,o,*} Detroit, Mich, San Francisco and Oakland, Calif, Minneapolis, Minn, Chicago, Ill, Denver, Colo, Mexico City, Mexico, and San Juan, Puerto Rico

Background: Inhaled corticosteroids (ICSs) are considered first-line treatment for persistent asthma, yet there is significant variability in treatment response. Dual-specificity phosphatase 1 (*DUSP1*) appears to mediate the anti-inflammatory action of corticosteroids.

Objective: We sought to determine whether variants in the *DUSP1* gene are associated with clinical response to ICS treatment.

Methods: Study participants with asthma were drawn from the following multiethnic cohorts: the Genetics of Asthma in Latino Americans (GALA) study; the Study of African Americans, Asthma, Genes & Environments (SAGE); and the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE). We screened GALA study participants for genetic variants that modified the relationship between ICS use and bronchodilator response. We then replicated our findings in SAGE and SAPPHIRE participants. In a group of SAPPHIRE participants treated with ICSs for 6 weeks, we examined whether a *DUSP1* polymorphism was associated with changes in FEV₁ and self-reported asthma control.

Results: The *DUSP1* polymorphisms rs881152 and rs34507926 localized to different haplotype blocks and appeared to significantly modify the relationship between ICS use and bronchodilator response among GALA study participants. This interaction was also seen for rs881152 among SAPPHIRE but not SAGE participants. Among the group of SAPPHIRE participants prospectively treated with ICSs for 6 weeks, rs881152 genotype was significantly associated with changes in self-reported asthma control but not FEV₁.

Conclusion: *DUSP1* polymorphisms were associated with clinical response to ICS therapy and therefore might be useful in the future to identify asthmatic patients more likely to respond to this controller treatment. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Asthma, inhaled corticosteroids, dual-specificity phosphatase 1, *DUSP1*, corticosteroid responsiveness

From ^athe Center for Health Services Research, Henry Ford Health System, Detroit; ^bWayne State University School of Medicine, Detroit; ^cthe Department of Medicine, University of California San Francisco, San Francisco General Hospital; ^dthe Department of Biostatistics and Research Epidemiology, Henry Ford Health System, Detroit; ^ethe Biomedical Genomics Center, University of Minnesota, Minneapolis; ^fChildren's Memorial Hospital, Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago; ^gthe Department of Medicine, National Jewish Medical and Research Center, Denver; ^hInstituto Nacional de Enfermedades Respiratorias, Mexico City; ⁱCentro de Neumología Pediátrica, CSP, San Juan; ^jthe James A. Watson Wellness Center, Oakland; ^kChildren's Hospital Oakland Research Institute, Oakland; ^lBay Area Pediatrics, Oakland; ^mVeterans Caribbean Health Care System, San Juan; ⁿthe Division of Allergy-Immunology, Northwestern University, Chicago; ^othe Department of Internal Medicine, Henry Ford Health System, Detroit; and ^pthe Department of Biopharmaceutical Sciences, University of California San Francisco.

*These authors contributed equally to this work.

Supported by grants from the American Asthma Foundation Strategic Program for Asthma Research, the National Institutes of Health (AI079139, AI061774, HL079055, and DK064695), and the Fund for Henry Ford Hospital to L. K. W. and the National Institutes of Health (HL078885, AI077439, HL088133, U01 GM61390, ES015794) and the Flight Attendant Medical Research Institute (FAMRI) to E. G. B.

Disclosure of potential conflict of interest: E. L. Peterson has received research support from the National Institutes of Health. R. Kumar has received research support from the National Institutes of Health/National Heart, Lung, and Blood Institute. J. R. Rodríguez-Santana has received research support from GlaxoSmithKline. W. Rodríguez-Cintrón has received research support from the University of California at San Francisco and Sociedad de Investigación Científica, Inc, and is a member of the Scientific Committee of the American College of Chest Physicians. P. C. Avila has received research support from Genentech/Roche. D. E. Lanfear has received research support from Episcource and the National Institutes of Health.

L. K. Williams has served on an advisory board for Merck & Co. The rest of the authors have declared that they have no conflict of interest.

Received for publication November 17, 2009; revised June 3, 2010; accepted for publication June 8, 2010.

Reprint requests: L. Keoki Williams, MD, MPH, Center for Health Services Research, Henry Ford Hospital, 1 Ford Place, 3A CHSR, Detroit, MI 48202. E-mail: kwillia5@hfhs.org.

0091-6749/\$36.00

© 2010 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2010.06.007

Inhaled corticosteroid (ICS) therapy is considered to be first-line treatment for the control of persistent asthma.¹ However, there appears to be substantial variation in ICS treatment response, which might contribute to a significant proportion of disease-related morbidity.²⁻⁴

In vitro models have identified dual-specificity phosphatase 1 (*DUSP1*), which is also known as mitogen-activated protein kinase (MAPK) phosphatase 1 (*MKPI*), as a potential master regulator of corticosteroid response.⁵⁻⁷ The induction of *DUSP1* activity by corticosteroids results in the dephosphorylation and

Abbreviations used

ACT:	Asthma Control Test
<i>DUSP1</i> :	Dual-specificity phosphatase 1
GALA:	Genetics of Asthma in Latino Americans
HFA:	Hydrofluoroalkane
ICS:	Inhaled corticosteroid
MAPK:	Mitogen-activated protein kinase
MDI:	Metered-dose inhaler
SAGE:	Study of African Americans, Asthma, Genes & Environments
SAPPHIRE:	Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity
SNP:	Single nucleotide polymorphism

inhibition of MAPKs, thereby reducing the expression and production of proinflammatory cytokines.⁸⁻¹³

Prior studies have shown bronchodilator responsiveness to be a consistent predictor of therapeutic response to ICS medication.¹⁴ Therefore in this study we assess whether single nucleotide polymorphisms (SNPs) in the *DUSP1* gene are associated with bronchodilator responsiveness in 3 separate, ethnically diverse cohorts and whether these relationships are modified by the concurrent use of ICS medication. We also assess whether implicated SNPs are prospectively associated with ICS treatment response among participants in the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE).

METHODS

Study population and setting

The Institutional Review Boards of Henry Ford Health System and the University of California, San Francisco, approved the study. Informed written consent was obtained from study participants or their guardians, and written assent was also obtained from all participants who were minors. Study participants comprised 3 separate cohorts, the Genetics of Asthma in Latino Americans (GALA) study; the Study of African Americans, Asthma, Genes & Environments (SAGE); and SAPPHIRE.

The first 2 studies, GALA and SAGE, were cross-sectional and have been described elsewhere.^{15,16} The GALA study began as 399 Puerto Rican and 301 Mexican asthmatic trios recruited from 4 clinical centers located in San Francisco, New York City, Puerto Rico, and Mexico City. SAGE included subjects recruited from the San Francisco Bay Area. Participants in both studies were 8 to 40 years old, had physician-diagnosed asthma or greater than 12% bronchodilator responsiveness, and had symptoms of coughing, wheezing, or shortness of breath for at least 2 years. Biological parents and grandparents were required to identify themselves as either Puerto Rican or Mexican American for GALA study participants and as African American for SAGE participants. Patients were excluded from these studies if they had a greater than 10 pack-year smoking history, smoked in the previous 12 months, or had a history of other lung diseases. Concurrent ICS use was assessed by means of questionnaire in these cohorts.

Subjects in the SAPPHIRE cohort all received care through a large health system serving southeast Michigan. Patients were invited for screening if they met the following criteria: age of 12 to 56 years, a prior clinical diagnosis of asthma, and no recorded diagnosis of chronic obstructive pulmonary disease or congestive heart failure. Longitudinal health care data were available for participants who had coverage through an affiliated provider. These data included pharmacy records for patients with prescription riders, which permitted assessment of concurrent ICS use in the larger group of screened participants.

Because SAPPHIRE was specifically designed to assess ICS responsiveness as one of its objectives, a subset of screened patients was invited to undergo 6 weeks of ICS treatment (ie, 160 µg of beclomethasone dipropionate hydrofluoroalkane [HFA; Teva Specialty Pharmaceuticals LLC, Horsham, Pa]

administered twice daily by means of metered-dose inhaler [MDI]), as has been done elsewhere.¹⁴ Participants had to meet the following criteria to qualify for this additional assessment: a baseline FEV₁ of between 40% and 90% of predicted value, bronchodilator reversibility (ie, improvement in FEV₁ of >12% from baseline value), no smoking in the preceding year or a less than 10 pack-year smoking history total, no oral or ICS use in the 4 weeks preceding enrollment, not pregnant at the time of enrollment, and no intention to become pregnant during the 6-week treatment period. Participants were instructed in proper MDI technique, including the use of a provided spacer (AeroChamber Plus Z STAT; Monahan Medical Corp, Plattsburgh, NY). All SAPPHIRE participants completed a survey at their baseline assessment, and subjects who underwent the 6 weeks of ICS treatment also completed a survey at the follow-up visit. Asthma control was assessed at each visit by using the Asthma Control Test (ACT; QualityMetric, Inc, Lincoln, RI).¹⁷

Assessment of pulmonary function

Spirometry was performed in accordance with 1995 American Thoracic Society¹⁸ and more recent 2005 American Thoracic Society/European Respiratory Society recommendations depending on the time of enrollment.^{19,20} A Fleisch-type pneumotachometer (KoKo PFT Spirometer; nSpire Health, Inc, Louisville, Colo) was used to measure pulmonary function parameters, and expected values were obtained by using standard equations derived from the US population.²¹ If possible, patients were asked to withhold their bronchodilator medications 12 hours before lung function tests. Bronchodilator response was determined by administering 360 µg of albuterol sulfate from a standard MDI, and a second dose (180 µg for children <16 years of age and 360 µg for persons ≥16 years of age) was administered if greater than 12% reversibility was not achieved after the first dose. A greater than 12% improvement in FEV₁ was considered reversible^{22,23} and was required for enrollment in the 6-week treatment trial. However, for the purposes of assessing ICS-by-genotype interactions on bronchodilator response, the difference in FEV₁ after the first dose of albuterol was used (ie, between the first and second set of spirometric measurements). Pulmonary function was reassessed after 6 weeks in the subset of subjects in the SAPPHIRE trial who received beclomethasone dipropionate HFA.

DNA isolation, genotyping, and assessment of ancestry

All study patients had genomic DNA extracted from whole blood samples. Genotyping was performed with iPLEX gold (Sequenom, Inc, San Diego, Calif). Each subject was genotyped for 107 SNPs, which were informative for determining subjects' ancestry because of their differing allele frequencies in continental population groups. This set of ancestry-informative markers has been described in detail elsewhere²⁴ but was developed from West African subjects living in London, United Kingdom, and South Carolina; subjects of European ancestry from Coriell's North American Caucasian panel (Coriell Institute for Medical Research, Camden, NJ); and Native American subjects (ie, Mayan and Nahua) from villages in Tlapa in the state of Guerrero, Mexico.

For the GALA study and SAGE cohorts, we estimated individual ancestry using the program STRUCTURE, which uses a Bayesian approach.²⁵ For the SAPPHIRE cohort, we used the software package PSMIX, which uses maximum likelihood estimation to estimate ancestry.^{26,27} We have previously shown that both methods produce highly correlated ancestry estimates.²⁸

Eight tagging SNPs were selected to provide coverage of the *DUSP1* gene (7 intronic and 1 synonymous), and 2 additional SNPs were selected that had minor allele frequencies of 5% or greater. This genotyping was performed by means of allelic discrimination with a Taqman SNP genotyping assay (Applied Biosystems, Foster City, Calif).

Statistical analysis

Given the reported association between bronchodilator responsiveness and ICS responsiveness,¹⁴ we first screened the *DUSP1* SNPs for association with bronchodilator responsiveness. We hypothesized that polymorphisms related to ICS responsiveness would demonstrate differential associations with the

TABLE I. Baseline characteristics of study subjects with asthma grouped according to the source cohort

Characteristic	Study cohorts			
	GALA study (n = 646)	SAGE (n = 264)	SAPPHIRE* (n = 430)	SAPPHIRE group treated with 6 wk of ICS therapy*† (n = 165)
Age (y), mean $\pm$ SD	15.2 $\pm$ 7.7	19.4 $\pm$ 9.3	30.9 $\pm$ 15.2	32.2 $\pm$ 13.5
Female sex, no. (%)	295 (45.7)	157 (59.5)	268 (62.3)	95 (57.6)
Latino ethnicity, no. (%)	646 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mexican	291 (45.0)	—	—	—
Puerto Rican	355 (55.0)	—	—	—
Self-reported race, no. (%)	—	—	—	—
Black	NA	264 (100.0)	430 (100.0)	165 (100.0)
White	NA	0 (0.0)	0 (0.0)	0 (0.0)
Other	NA	0 (0.0)	0 (0.0)	0 (0.0)
Proportion of ancestral admixture (%), mean $\pm$ SD	—	—	—	—
African	20.5 $\pm$ 10.8	79.2 $\pm$ 10.6	77.8 $\pm$ 10.0	78.9 $\pm$ 10.7
European	48.8 $\pm$ 16.9	17.0 $\pm$ 9.5	18.7 $\pm$ 10.0	17.1 $\pm$ 10.3
Native American	30.7 $\pm$ 20.7	3.8 $\pm$ 4.0	3.5 $\pm$ 3.5	2.7 $\pm$ 3.4
Duration of asthma (y), mean $\pm$ SD	8.8 $\pm$ 5.9	12.2 $\pm$ 8.4	17.5 $\pm$ 12.4	20.7 $\pm$ 12.6
ICS use at baseline, no. (%)	369 (57.1)	144 (54.5)	45 (10.5)	90 (5.8)
FEV ₁ % predicted at baseline, mean $\pm$ SD	85.9 $\pm$ 17.4	92.5 $\pm$ 16.4	89.5 $\pm$ 17.4	76.1 $\pm$ 17.4
FEV ₁ % reversibility at baseline, mean $\pm$ SD	8.2 $\pm$ 12.8	9.2 $\pm$ 9.7	10.6 $\pm$ 11.9	21.4 $\pm$ 12.3
ACT score at baseline, mean $\pm$ SD	—	—	19.8 $\pm$ 4.3	17.8 $\pm$ 5.1

NA, Not available.

*The SAPPHIRE cohort (n = 430) analytic set was restricted to those who members of the health plan and who reported African American race-ethnicity. This number included 48 (29%) of the 165 subjects who subsequently were enrolled in the 6-week ICS treatment trial.

†The treatment group included African American patients with asthma who qualified for and consented to 6 weeks of beclomethasone dipropionate HFA treatment.

bronchodilator responsiveness between subjects currently treated and those not treated with an inhaled steroid. Alternatively stated, given the biologic association between ICS use and β -agonist response,^{29,30} genetic variants (ie, SNP genotypes) that mediate steroid response should therefore demonstrate differing patterns of association with bronchodilator response in the presence and absence of ICS use. Accordingly, we assessed for effect modification by ICS use (ie, genotype-by-steroid use interactions on bronchodilator response). The dependent variable, bronchodilator responsiveness, was measured as the percentage change between the prebronchodilator and postbronchodilator FEV₁. Because our analysis involved an assessment of ICS-by-genotype interactions, we restricted the study population to just subjects with asthma in all cohorts (ie, case-only analysis).

We first assessed for significant associations between both genotype and genotype-by-ICS use interaction term on bronchodilator responsiveness in the GALA study cohort using multivariable linear regression models. An additive (or codominant) genetic model was used for the initial screening of each genotype. In other words, heterozygotic subjects were assumed to have a quantitative phenotype halfway between those of homozygotic subjects (ie, the SNP genotypes were coded as 0, 1, and 2), as has been done elsewhere.^{31,32} These regression models also included potential confounders, such as age, sex, proportion of African ancestry, proportion of Native American ancestry, duration of asthma, and study recruitment site. Among the GALA study cohort, we also stratified by Puerto Rican and Mexican ethnicity because these groups have previously shown pharmacogenetic differences in drug responsiveness.^{33,34} Polymorphisms that demonstrated significant direct and interaction associations with bronchodilator responsiveness were reassessed in the SAGE and SAPPHIRE cohorts (this SAPPHIRE group included only subjects enrolled in the health plan and in whom we have previously demonstrated near complete capture of ICS use).³⁵ We again assessed for significant associations between both genotype and genotype-by-ICS interaction term on bronchodilator responsiveness, adjusting for age, sex, proportion of African ancestry, proportion of Native American ancestry, and duration of asthma. Models for the SAGE cohort included an additional covariate for study recruitment site, whereas all SAPPHIRE subjects were recruited from a single health system. Based on the plot of these relations by rs881152 genotype, we also assessed a dominant/recessive genetic model for this genotype (ie, GG vs AG and AA) in the GALA study, SAGE, and SAPPHIRE cohorts.

We assessed the relationship between rs881152 genotype and ICS responsiveness in the subgroup of SAPPHIRE participants treated with 6 weeks of beclomethasone therapy. ICS responsiveness was defined as both the change in FEV₁ between the baseline visit and after 6 weeks of ICS therapy and as the change in self-reported asthma control between baseline and follow-up (ie, the change in ACT score after 6 weeks of treatment).

The Pearson χ^2 test was used to assess for deviations from Hardy-Weinberg equilibrium. Assessments of linkage disequilibrium between SNPs and haplotype block analyses were performed with Haploview 4.1, which is available at <http://www.broad.mit.edu/mpg/haploview>.³⁶ The outcome variables used in the regression analyses (ie, bronchodilator response, percentage change in FEV₁, and change in ACT score) were assessed for their distribution. All of these distributions satisfied the assumption of normality and were therefore used in the regression analyses without additional transformations. Analyses were performed with SAS version 9.1 (SAS Institute, Inc, Cary, NC)³⁷ and R version 2.9.0.³⁸

RESULTS

Study populations

The baseline characteristics of subjects with asthma in the 4 cohorts, the GALA study, SAGE, SAPPHIRE, and the SAPPHIRE treatment subgroup, are shown in Table I. Subjects in the GALA study and SAGE were younger when compared with the 2 SAPPHIRE groups, with average ages of 15.2, 19.4, 30.9, and 32.2 years, respectively. All participants in the GALA study were of Latino ethnicity, with 291 (45.0%) considering themselves Mexican and 355 (55.0%) considering themselves Puerto Rican. In contrast, all of the subjects from the SAGE and SAPPHIRE cohorts self-identified as non-Hispanic, African American. Likewise, the average proportion of genetic African ancestry in the SAGE participants and the 2 SAPPHIRE groups was 79.2%, 77.8%, and 78.9%, respectively. The distribution of genetic ancestry among the entire GALA study cohort was 20.5% African, 48.8% European, and 30.7% Native American. However, this distribution differed between Mexican and Puerto

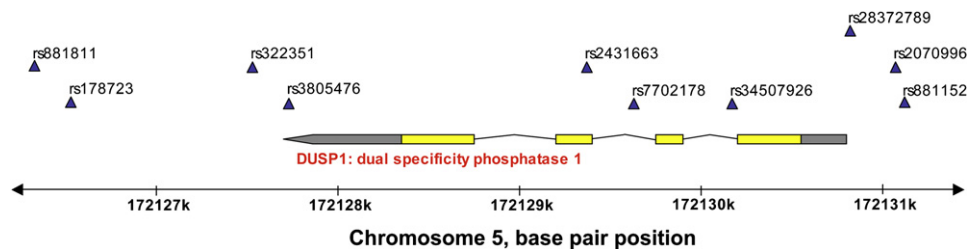


FIG 1. Chromosomal and *DUSP1* position of SNPs assessed. Polymorphism positions are indicated with blue triangles. Exons are shown in yellow, untranslated regions in gray, and introns as connecting lines. The 3' untranslated region is denoted by the pointed end.

TABLE II. *DUSP1* SNPs that modify the relationship between ICS use and bronchodilator responsiveness among GALA study participants with asthma*

SNP no.	Polymorphism†	All patients (n = 646)			Mexican American patients (n = 291)			Puerto Rican patients (n = 355)		
		Minor allele frequency	Genotype associated P value	Genotype × ICS use interaction P value	Minor allele frequency	Genotype associated P value	Genotype × ICS use interaction P value	Minor allele frequency	Genotype associated P value	Genotype × ICS use interaction P value
rs881152	G/A	0.14	.021	.100	0.14	.744	.886	0.14	.004	.026
rs2070996	C/T	0.22	.391	.633	0.27	.667	.789	0.25	.445	.373
rs28372789	A/–‡	0.38	.753	.654	0.36	.347	.408	0.40	.202	.176
rs34507926	C/G	0.11	.090	.594	0.15	.307	.102	0.08	<.001	.005
rs7702178	T/C	0.21	.050	.256	0.24	.760	.886	0.18	.005	.041
rs2431663	T/G	0.07	.142	.579	0.04	.980	.438	0.10	.109	.274
rs3805476	G/A	0.21	.057	.257	0.25	.646	.960	0.18	.009	.043
rs322351	C/T	0.39	.517	.522	0.37	.361	.416	0.42	.102	.123
rs178723	A/G	0.44	.886	.755	0.45	.865	.880	0.43	.775	.973
rs881811	C/T	0.14	.635	.830	0.16	.257	.103	0.13	.043	.115

*The linear regression equations used were of the following form: ΔFEV_1 (% Change postbronchodilator vs prebronchodilator) = $\alpha + \beta_1(\text{Genotype}) + \beta_2(\text{Current ICS use}) + \beta_3(\text{Genotype} \times \text{ICS use}) + \beta_4(\text{Age}) + \beta_5(\text{Sex}) + \beta_6(\text{Proportion of African ancestry}) + \beta_7(\text{Proportion of Native American ancestry}) + \beta_8(\text{Duration of asthma}) + \beta_9(\text{Study recruitment site}) + \text{Error}$. An additive genetic model was used for each genotype. The *P* values for the genotype coefficient (β_1) and the genotype-by-ICS use interaction term (β_3) are presented in the table.

†Presented in the format of major allele/minor allele.

‡Deletion polymorphism.

Rican subjects in the GALA study, as previously reported.³⁹ Among Mexican subjects, the average proportion of African, European, and Native American ancestry was 13.1%, 37.7%, and 49.2%, respectively. Among Puerto Rican subjects, the average proportion of African, European, and Native American ancestry was 26.6%, 57.9%, and 15.5%, respectively.

Screening for *DUSP1* genotypes that modify the relationship between ICS use and bronchodilator responsiveness

Given the ancestral diversity of the GALA study participants, we first used this population to screen for associations between *DUSP1* genotypes and bronchodilator responsiveness. In particular, we assessed for significant interactions between these genotypes and concurrent ICS use (ie, to assess which *DUSP1* polymorphisms appeared to affect the relationship between ICS use and bronchodilator response) while adjusting for age, sex, proportion of African ancestry, proportion of Native American ancestry, duration of asthma, and study recruitment site. The location of these polymorphisms on chromosome 5 and their relationship to *DUSP1* are shown in Fig 1. Assessments of Hardy-Weinberg equilibrium are shown in Table E1 of this article's Online Repository at www.jacionline.org.

Among the entire GALA study cohort, only 1 SNP, rs881152, was significantly associated with bronchodilator responsiveness,

and none showed a significant genotype-ICS interaction (Table II). However, after stratifying by ethnicity, we observed a number of significant associations among Puerto Rican patients with asthma but none among the Mexican patients. In particular, the SNPs rs881152, rs34507926, rs7702178, and rs3805476 showed both significant genotype and ICS interaction associations with bronchodilator responsiveness.

Linkage disequilibrium for the *DUSP1* SNPs and their estimated haplotype blocks in the GALA study cohort are shown in Fig E1 (available in this article's Online Repository at www.jacionline.org). Among the Puerto Rican participants, SNPs rs34507926, rs7702178, and rs3805476, which demonstrated the aforementioned significant ICS interactions, were attributed to the same haplotype block (see Fig E1, C). Therefore for replication in SAGE and SAPHIRE, we selected the most significant SNP, rs34507926, in this group for further analysis. We also selected SNP rs881152 for further analysis because it represented different haplotype blocks in Mexicans and Puerto Ricans.

Replication of *DUSP1* genotypes that modify the relationship between ICS use and bronchodilator responsiveness

Replication of rs881152 and rs34507926 in the SAGE and SAPHIRE cohorts is shown in Table III. In both cohorts we observed a consistent relationship between ICS use and

TABLE III. Replication of interactions between *DUSP1* genotypes and ICS use on baseline bronchodilator responsiveness among patients with asthma (SAGE [n = 264] and SAPHIRE [n = 430] cohorts)*

Covariates	SAGE cohort						SAPHIRE cohort					
	Model 1†		Model 2‡		Model 3§		Model 1†		Model 2‡		Model 3§	
	Bronchodilator % change in FEV ₁	P value	Bronchodilator % change in FEV ₁	P value	Bronchodilator % change in FEV ₁	P value	Bronchodilator % change in FEV ₁	P value	Bronchodilator % change in FEV ₁	P value	Bronchodilator % change in FEV ₁	P value
rs881152 genotype	−0.08	.936	−0.56	.722	−1.28	.432	−0.46	.635	0.19	.851	0.31	.770
ICS use	2.813	.030	2.45	.122	2.99	.069	3.96	.037	7.00	.004	7.58	.003
rs881152 genotype by ICS interaction	—	—	0.847	.685	1.29	.554	—	—	−6.55	.044	−6.72	.043
rs34507926 genotype	1.49	.535	3.34	.366	7.97	.042	3.50	.118	3.45	.138	4.29	.077
ICS use	2.37	.036	2.53	.029	2.83	.020	4.18	.028	4.14	.033	4.53	.025
rs34507926 genotype by ICS interaction	—	—	−3.21	.509	−6.38	.191	—	—	0.84	.926	1.27	.888

*An additive genetic model was used for each genotype. The genotypes for rs881152 GG, AG, and AA, were coded as 0, 1, and 2, respectively. The genotypes for rs34507926 CC, CG, and GG, were coded as 0, 1, and 2, respectively. However, there were no subjects with the rs34507926 genotype GG in the SAPHIRE analytic group, such that CC and CG were coded as 0 and 1, respectively, for this analysis.

†Model 1 follows the form ΔFEV_1 (% Change postbronchodilator vs prebronchodilator) = $\alpha + \beta_1(\text{Genotype}) + \beta_2(\text{Current ICS use}) + \text{Error}$.

‡Model 2 follows the form ΔFEV_1 (% Change postbronchodilator vs prebronchodilator) = $\alpha + \beta_1(\text{Genotype}) + \beta_2(\text{Current ICS use}) + \beta_3(\text{Genotype} \times \text{ICS use}) + \text{Error}$.

§Model 3 follows the form ΔFEV_1 (% Change postbronchodilator vs prebronchodilator) = $\alpha + \beta_1(\text{Genotype}) + \beta_2(\text{Current ICS use}) + \beta_3(\text{Genotype} \times \text{ICS use}) + \beta_4(\text{Age}) + \beta_5(\text{Sex}) + \beta_6(\text{Proportion of African ancestry}) + \beta_7(\text{Proportion of Native American ancestry}) + \beta_8(\text{Duration of asthma}) + \text{Error}$. An additional term for study recruitment site was added for the SAGE cohort, but this term was not included for the SAPHIRE because since all subjects in the latter group were enrolled from a single, large health system. The parameter estimates for the first 3 terms are shown in the table.

bronchodilator responsiveness. However, only in the SAPHIRE cohort did genotype rs881152 appear to modify the relationship between ICS use and bronchodilator responsiveness (the magnitude and direction of interaction estimate was similar to that seen in the GALA study, data not shown). The negative interaction term in SAPHIRE implied that among those taking inhaled steroids, the subjects with the GG genotype had greater bronchodilator response than those with the AA genotype ($P = .044$ and $P = .043$ in models 2 and 3, respectively). This is shown graphically in Fig 2. Fig 2 also suggests that the relationship might be dominant/recessive, with the GG genotype subjects showing the greatest bronchodilator response in the setting of ICS use. Reassessing the ICS-by-rs881152 genotype interaction with this relationship (ie, GG vs AG and AA) strengthens the interaction in all of the GALA study participants ($P = .060$), the subset of Puerto Rican participants in the GALA study ($P = .014$), and the SAPHIRE participants ($P = .039$, data not shown).

In contrast, the rs34507926 genotype was significantly associated with bronchodilator responsiveness in the SAGE cohort ($P = .042$), although not in the SAPHIRE cohort ($P = .143$), after adjusting for potential confounders (Table III). The interaction term estimate for rs34507926 genotype and ICS use did not reach statistical significance in any of the models.

Assessing the relationship between *DUSP1* genotype rs881152 and steroid response

Because the aforementioned significant interactions suggested that these *DUSP1* polymorphisms might affect the response to ICS medication, we specifically assessed this relationship in a separate subgroup of SAPHIRE participants treated for 6 weeks with beclomethasone dipropionate HFA. Here the outcome was the percentage improvement in prebronchodilator FEV₁ over the 6-week treatment period and the change in self-reported asthma control. The rs881152 genotype was not associated with a change in FEV₁ over 6 weeks of ICS treatment (Table IV and

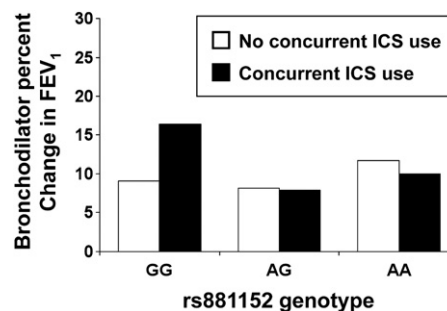


FIG 2. Relationship between *DUSP1* genotype rs881152 and bronchodilator responsiveness among subjects in the SAPHIRE cohort stratified by ICS use. Bronchodilator responsiveness was measured as the percentage change in prebronchodilator and postbronchodilator FEV₁ at the initial screening visit for SAPHIRE subjects. Concurrent ICS use at the time of this screening was determined from patient report and from pharmacy claims. The numbers of subjects with the rs881152 genotypes GG, AG, and AA were 239, 125, and 21, respectively, among those without concurrent ICS use and 26, 17, and 2, respectively, among those currently using ICS medication.

Fig 3, A). However, the *DUSP1* genotype at rs881152 was significantly associated with a change in self-reported asthma control over the 6-week ICS treatment period. Here asthma control was measured by using the ACT and the change in control as the absolute change in this measure. As shown in Fig 3, B, the mean change in ACT by rs881152 genotype was 2.81 (± 6.22 SD) for GG, 1.38 (± 7.36 SD) for AG, and -3.5 (± 9.13 SD) for AA. The trend across rs881152 genotypes was statistically significant before and after adjusting for potential confounders ($P = .011$ and $P = .013$, respectively; Table IV).

We also performed the following *post hoc* analyses. First, the relationship between asthma control and a dominant/recessive genetic model for rs881152 genotype (ie, GG vs AG and AA) produced results of borderline statistical significance ($P = .060$ and $P = .085$ for the unadjusted and adjusted models, respectively;

TABLE IV. Relationship between *DUSP1* rs881152 genotype and both change in pulmonary function and self-reported asthma control after 6 weeks of ICS use among patients with asthma (SAPPHIRE treatment group [*n* = 165])*

Variable	Percentage change in FEV ₁ from baseline†				Change in ACT score from baseline‡			
	Unadjusted mean ± SD	<i>P</i> value	Adjusted mean ± SD§	<i>P</i> value	Unadjusted mean ± SD	<i>P</i> value	Adjusted mean ± SD§	<i>P</i> value
rs881152 genotype	−0.05 ± 2.06	.981	−0.42 ± 1.78	.812	−2.28 ± 0.89	.011	−2.21 ± 0.88	.013

*The SAPPHIRE subgroup are those patients with asthma who were eligible and agreeable to 6 weeks of treatment with inhaled beclomethasone dipropionate HFA. An additive genetic model was used for the rs881152 genotype, whereby GG = 0, AG = 1, and AA = 2.

†The percentage change in FEV₁ was measured as a difference between the prebronchodilator FEV₁ measurements at the initial visit and the 6-week follow-up visit by using the following equation: ((FEV₁ (6-week follow-up) − FEV₁ (Initial))/FEV₁ (Initial)) × 100%.

‡The ACT has a score range of 5 to 25, with higher scores signifying better control. Therefore a negative coefficient suggests a worsening of control between baseline and the 6-week follow-up examination.

§Adjusted for patient age, sex, duration of asthma, and baseline percent predicted FEV₁.

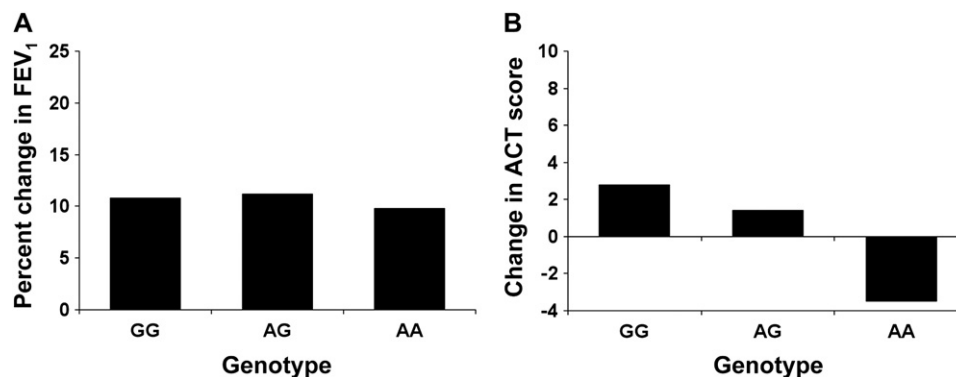


FIG 3. Relationship between *DUSP1* genotype rs881152 and ICS responsiveness among the subgroup of subjects treated as part of SAPPHIRE. ICS responsiveness was measured both as the percentage change in prebronchodilator FEV₁ (**A**) and the change in asthma control (**B**) between the initial screening visit and after 6 weeks of ICS treatment. The change in asthma control was measured as the absolute difference in ACT score at these 2 time points. The numbers of subjects with rs881152 genotypes GG, AG, and AA were 88, 63, and 10, respectively, for the analysis looking at percentage change in prebronchodilator FEV₁. The numbers of subjects with rs881152 genotypes GG, AG, and AA were 86, 63, and 10, respectively, for the analysis looking at the change in ACT score. As shown in Table IV, the *P* values for the association between rs881152 genotype (ie, coded GG = 0, AG = 1, and AA = 2) and both FEV₁ change (Fig 3, A) and ACT score change (Fig 3, B) were 0.981 and 0.011, respectively.

data not shown). Second, there was no relationship between rs881152 genotype and change in bronchodilator responsiveness over the 6-week ICS treatment period (data not shown).

DISCUSSION

Here we report a relationship between polymorphisms in *DUSP1* and response to ICSs. To identify risk alleles, we took a staged approach, first screening for polymorphisms that appeared to affect the cross-sectional relationship between ICS use and bronchodilator response and then validating in additional cohorts, followed by confirmation in a prospective study of ICS responsiveness.

The rationale for this approach is that patients with steroid-resistant asthma might experience greater proteolytic and inflammatory activity, leading to airway remodeling and decreased bronchodilator response.⁴⁰ Furthermore, an increased inflammatory milieu, as found in patients with steroid-resistant asthma, might reduce airway smooth muscle relaxation by inhibiting cyclic AMP production or by directly inhibiting β_2 -receptor-mediated gene expression.⁴¹ Corticosteroids can enhance β_2 -agonist-induced cyclic AMP production²⁹ and have been

shown to prevent inflammatory cytokine-mediated uncoupling of β_2 -receptor activation of its downstream targets.³⁰ Together, these observations provide a potential mechanism whereby polymorphisms that appear to modify the relationship between ICS use and bronchodilator response might also influence steroid responsiveness as measured more directly (eg, as an improvement in pulmonary function or self-reported asthma control). As such, our novel approach might prove useful to screen for other polymorphisms associated with steroid response.

Corticosteroids induce the expression and prevent the proteolytic degradation of *DUSP1*, a key inhibitor of the MAPK cascade.^{42,43} For example, Kang et al¹¹ found that dexamethasone is able to induce *DUSP1* and subsequently decrease c-Jun N-terminal kinase and p38 MAPK activity in human airway smooth muscle. Conversely, in murine models corticosteroid inhibition of c-Jun N-terminal kinase and p38 MAPK is impaired in *DUSP1*^{-/-} macrophages,⁸ and these cells produce excessive amounts of inflammatory cytokines, including TNF- α and IL-6.⁴⁴ Bhavsar et al⁴⁵ found increased activation of p38 MAPK in airway macrophages from patients with severe asthma when compared with patients with nonsevere asthma and the severe asthmatic group also exhibited impaired corticosteroid induction of

DUSP1. Therefore although not previously examined, our observation of a relationship between *DUSP1* polymorphisms and ICS-related improvement in self-reported asthma control is not altogether surprising.

Nevertheless, we do not currently know whether the polymorphisms identified here affect *DUSP1* function or whether they are just in linkage disequilibrium with genetic variants that do. In particular, rs881152, which was most significantly associated with ICS response, is located in the promoter region of *DUSP1* (ie, 357 bp proximal to the transcription start site). This was the most 5' SNP that we investigated in this study. However, a recent study by Johansson-Haque et al⁴⁶ suggests that the promoter site involved in glucocorticoid-induced gene expression might be further upstream, and Tchen et al⁴⁷ have identified glucocorticoid-responsive regions approximately 1.3 and 4.6 kb upstream of the *DUSP1* transcription start site. Therefore it is possible that polymorphisms upstream of those identified here might have even stronger associations with clinical ICS response than we demonstrated.

This study must be interpreted in light of its other limitations as well. First, the observed associations were not consistent across all of the racial-ethnic groups studied. For example, the gene-drug interactions observed in Puerto Rican patients with asthma were not observed in Mexican subjects. However, we have previously observed different pharmacologic and pharmacogenetic interactions between these 2 ethnic groups.^{16,33,48} Although we adjusted for individual genetic ancestry in our models, it is still possible that the between-group differences that we observed were due to additional gene-gene or gene-environment interactions that were specific to these groups or their areas of residence. In addition, different patterns of linkage disequilibrium between the polymorphisms examined here and the causal variants could have resulted in between-group differences. Moreover, concurrent ICS use was determined by patient report in the GALA study and SAGE cohorts but was assessed by actual pharmacy fills in SAPHIRE. This might account in part for the differences in estimated ICS use at baseline between the GALA study, SAGE, and SAPHIRE cohorts, and it might also explain some of the between-group differences in pharmacogenetic interactions.

We assessed genotype-by-ICS interactions in the GALA study cohort without accounting for multiple comparisons. We believed that this was a reasonable approach given the underlying linkage disequilibrium between polymorphisms in *DUSP1* (see Fig E1). Although the appropriate significance threshold for interactive effects is less well defined, a principal component approach can be used to account for correlation between SNPs.⁴⁹ Applying this approach suggests that the threshold for genotype significance in the GALA study should be downwardly adjusted by a factor of 5 (ie, $P < .01$; data not shown). Therefore the genotype effects for the SNPs promoted for replication in SAGE and SAPHIRE still met this threshold. Moreover, the significant interaction for rs881152 was replicated in SAPHIRE, providing further evidence for this relationship.

It is also uncertain which genetic model is most appropriate for SNP rs881152 (ie, codominate vs dominant/recessive) because both appeared plausible in our analyses. This will require further functional analysis of this genetic variant. Lastly, among participants in the treatment trial, we noted a prospective difference in self-reported asthma control by rs881152 genotype, which was not accompanied by a commensurate change in pulmonary function. This unlinking of symptoms and physiologic measures

might be due in part to the short treatment period; however, an even longer prospective study of ICS use noted symptomatic improvements without significant changes in FEV₁.⁵⁰

In conclusion, we demonstrate here that a *DUSP1* polymorphism that appears to modify the relationship between steroid use and bronchodilator response is also associated with a symptomatic response to ICS therapy (ie, a change in ACT score). Our findings suggest that this polymorphism (or one in linkage disequilibrium) might be useful in identifying asthmatic patients more likely to respond to ICS controller treatment. However, the between-group differences that we observed suggest that additional genetic and environmental effects might exist that deserve further study.

We thank the families and their patients for their participation. We also thank the numerous health care providers and community clinics for their support and participation in the GALA study, SAGE, and SAPHIRE. We especially thank Jeffrey M. Drazen, MD; Scott Weiss, MD; Ed Silverman, MD, PhD; Homer A. Boushey, MD; and Jean G. Ford, MD, for their efforts toward the creation of the GALA study.

Clinical implications: These findings further our understanding of ICS pharmacogenetics and will hopefully result in improved tailoring of this controller therapy among subjects with asthma and better disease control.

REFERENCES

- Expert Panel Report 3 (EPR-3): guidelines for the diagnosis and management of asthma—summary report 2007. *J Allergy Clin Immunol* 2007;120(suppl):S94-138.
- Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. *Lancet* 2009;373:1905-17.
- Sobande PO, Kerckmar CM. Inhaled corticosteroids in asthma management. *Respir Care* 2008;53:625-33.
- Szefer SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002;109:410-8.
- Abraham SM, Clark AR. Dual-specificity phosphatase 1: a critical regulator of innate immune responses. *Biochem Soc Trans* 2006;34:1018-23.
- Clark AR. MAP kinase phosphatase 1: a novel mediator of biological effects of glucocorticoids? *J Endocrinol* 2003;178:5-12.
- Clark AR, Lasa M. Crosstalk between glucocorticoids and mitogen-activated protein kinase signalling pathways. *Curr Opin Pharmacol* 2003;3:404-11.
- Abraham SM, Lawrence T, Kleiman A, Warden P, Medghalchi M, Tuckermann J, et al. Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *J Exp Med* 2006;203:1883-9.
- Bhavsar P, Ahmad T, Adcock IM. The role of histone deacetylases in asthma and allergic diseases. *J Allergy Clin Immunol* 2008;121:580-4.
- Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 2002;20:55-72.
- Kang BN, Jude JA, Panettieri RA Jr, Walseth TF, Kannan MS. Glucocorticoid regulation of CD38 expression in human airway smooth muscle cells: role of dual specificity phosphatase 1. *Am J Physiol Lung Cell Mol Physiol* 2008;295:L186-93.
- Pelaia G, Cuda G, Vatrella A, Gallelli L, Caraglia M, Marra M, et al. Mitogen-activated protein kinases and asthma. *J Cell Physiol* 2005;202:642-53.
- Zhao Q, Shepherd EG, Manson ME, Nelin LD, Sorokin A, Liu Y. The role of mitogen-activated protein kinase phosphatase-1 in the response of alveolar macrophages to lipopolysaccharide: attenuation of proinflammatory cytokine biosynthesis via feedback control of p38. *J Biol Chem* 2005;280:8101-8.
- Martin RJ, Szefer SJ, King TS, Kraft M, Boushey HA, Chinchilli VM, et al. The Predicting Response to Inhaled Corticosteroid Efficacy (PRICE) trial. *J Allergy Clin Immunol* 2007;119:73-80.
- Tsai HJ, Kho JY, Shaikh N, Choudhry S, Naqvi M, Navarro D, et al. Admixture-matched case-control study: a practical approach for genetic association studies in admixed populations. *Hum Genet* 2006;118:626-39.
- Naqvi M, Tcheurekdjian H, DeBoard JA, Williams LK, Navarro D, Castro RA, et al. Inhaled corticosteroids and augmented bronchodilator responsiveness in Latino and African American asthmatic patients. *Ann Allergy Asthma Immunol* 2008;100:551-7.

17. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59-65.
18. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;152:1107-36.
19. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319-38.
20. Miller MR, Crapo R, Hankinson J, Brusasco V, Burgos F, Casaburi R, et al. General considerations for lung function testing. *Eur Respir J* 2005;26:153-61.
21. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;159:179-87.
22. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991;144:1202-18.
23. National Heart, Lung, and Blood Institute. Global Initiative for Asthma: global strategy for asthma management and prevention. Bethesda (MD): National Heart, Lung, and Blood Institute; 2002. Publication no. 02-3659.
24. Yaeger R, Avila-Bront A, Abdul K, Nolan PC, Grann VR, Burchette MG, et al. Comparing genetic ancestry and self-described race in African Americans born in the United States and in Africa. *Cancer Epidemiol Biomarkers Prev* 2008;17:1329-38.
25. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;155:945-59.
26. Wu B, Liu N, Zhao H. PSMIX: an R package for population structure inference via maximum likelihood method. *BMC Bioinformatics* 2006;7:317.
27. Tang H, Peng J, Wang P, Risch NJ. Estimation of individual admixture: analytical and study design considerations. *Genet Epidemiol* 2005;28:289-301.
28. Yang JJ, Burchard EG, Choudhry S, Johnson CC, Ownby DR, Favro D, et al. Differences in allergic sensitization by self-reported race and genetic ancestry. *J Allergy Clin Immunol* 2008;122:820-7.
29. Aksoy MO, Mardini IA, Yang Y, Bin W, Zhou S, Kelsen SG. Glucocorticoid effects on the beta-adrenergic receptor-adenylyl cyclase system of human airway epithelium. *J Allergy Clin Immunol* 2002;109:491-7.
30. Mak JC, Hisada T, Salmon M, Barnes PJ, Chung KF. Glucocorticoids reverse IL-1beta-induced impairment of beta-adrenoceptor-mediated relaxation and up-regulation of G-protein-coupled receptor kinases. *Br J Pharmacol* 2002;135:987-96.
31. Zeggini E, Scott LJ, Saxena R, Voight BF, Marchini JL, Hu T, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 2008;40:638-45.
32. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009;41:25-34.
33. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J, et al. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. *Am J Respir Crit Care Med* 2005;171:563-70.
34. Naqvi M, Thyne S, Choudhry S, Tsai HJ, Navarro D, Castro RA, et al. Ethnic-specific differences in bronchodilator responsiveness among African Americans, Puerto Ricans, and Mexicans with asthma. *J Asthma* 2007;44:639-48.
35. Williams LK, Joseph CL, Peterson EL, Wells K, Wang M, Chowdhry VK, et al. Patients with asthma who do not fill their inhaled corticosteroids: a study of primary nonadherence. *J Allergy Clin Immunol* 2007;120:1153-9.
36. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263-5.
37. SAS Institute, Inc. SAS/STAT users guide. Version 9.1. Cary (NC): SAS Institute, Inc; 2004.
38. R Development Core Team. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2008.
39. Choudhry S, Coyle NE, Tang H, Salari K, Lind D, Clark SL, et al. Population stratification confounds genetic association studies among Latinos. *Hum Genet* 2006;118:652-64.
40. Goleva E, Hauk PJ, Boguniewicz J, Martin RJ, Leung DY. Airway remodeling and lack of bronchodilator response in steroid-resistant asthma. *J Allergy Clin Immunol* 2007;120:1065-72.
41. Shore SA, Moore PE. Regulation of beta-adrenergic responses in airway smooth muscle. *Respir Physiol Neurobiol* 2003;137:179-95.
42. Lasa M, Abraham SM, Boucheron C, Saklatvala J, Clark AR. Dexamethasone causes sustained expression of mitogen-activated protein kinase (MAPK) phosphatase 1 and phosphatase-mediated inhibition of MAPK p38. *Mol Cell Biol* 2002;22:7802-11.
43. Kassel O, Sancono A, Kratzschmar J, Kreft B, Stassen M, Cato AC. Glucocorticoids inhibit MAP kinase via increased expression and decreased degradation of MKP-1. *EMBO J* 2001;20:7108-16.
44. Dickinson RJ, Keyse SM. Diverse physiological functions for dual-specificity MAP kinase phosphatases. *J Cell Sci* 2006;119:4607-15.
45. Bhavsar P, Hew M, Khorasani N, Torrego A, Barnes PJ, Adcock I, et al. Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared with non-severe asthma. *Thorax* 2008;63:784-90.
46. Johansson-Haque K, Palanichamy E, Okret S. Stimulation of MAPK-phosphatase 1 gene expression by glucocorticoids occurs through a tethering mechanism involving C/EBP. *J Mol Endocrinol* 2008;41:239-49.
47. Tchen CR, Martins JR, Paktiawal N, Perelli R, Saklatvala J, Clark AR. Glucocorticoid regulation of mouse and human dual specificity phosphatase 1 (DUSP1) genes: unusual cis-acting elements and unexpected evolutionary divergence. *J Biol Chem* 2010;285:2642-52.
48. Tcheurekdjian H, Thyne SM, Williams LK, Via M, Rodriguez-Santana JR, Rodriguez-Cintrón W, et al. Augmentation of bronchodilator responsiveness by leukotriene modifiers in Puerto Rican and Mexican children. *Ann Allergy Asthma Immunol* 2009;102:510-7.
49. Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008;32:361-9.
50. Childhood Asthma Management Program Research Group. Long-term effects of budesonide or nedocromil in children with asthma. *N Engl J Med* 2000;343:1054-63.

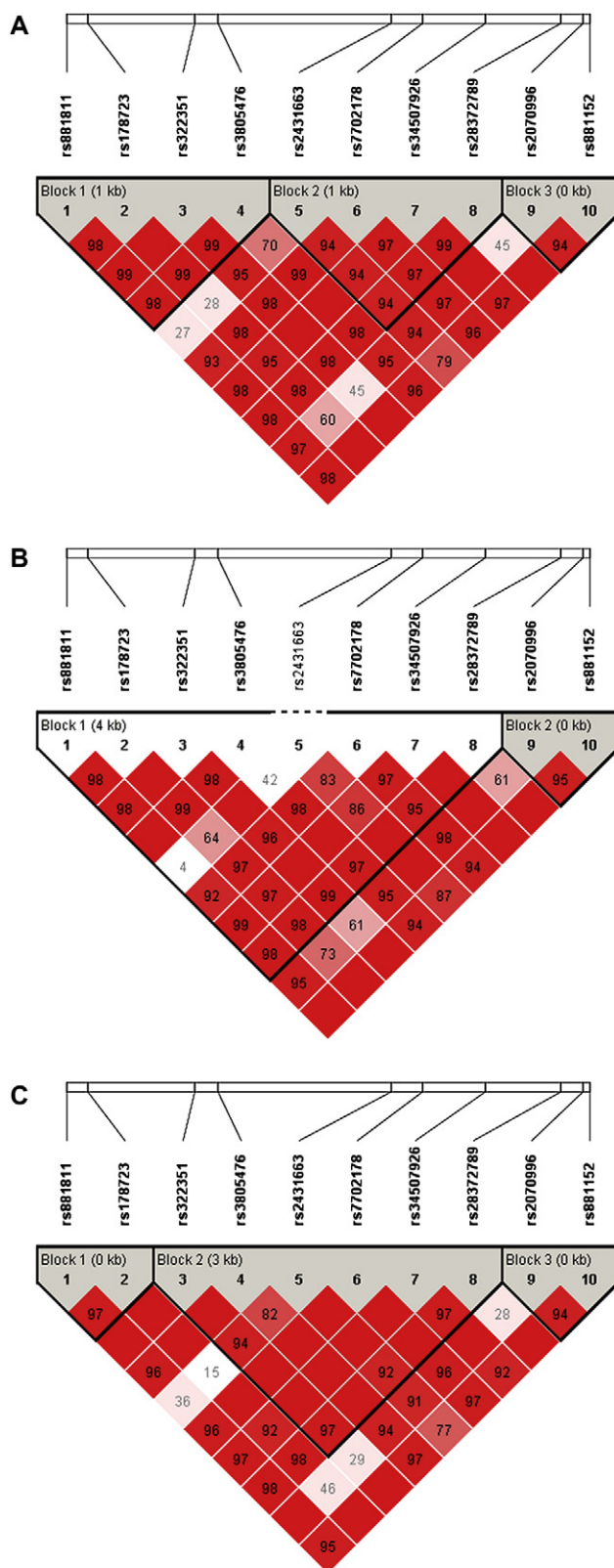


FIG E1. Linkage disequilibrium between *DUSP1* SNPs in the GALA cohort stratified for Mexican American and Puerto Rican patients with asthma. Both linkage disequilibrium between the polymorphisms and haplotype blocks for *DUSP1* are shown for subjects in the GALA study cohort with asthma (**A**) and after stratifying this group for Mexican American (**B**) and Puerto Rican (**C**) ethnicity. The D' statistic is presented for the linkage disequilibrium between polymorphisms.

TABLE E1. Assessment of Hardy-Weinberg equilibrium for the *DUSP1* polymorphisms examined in the study cohorts*

SNP	Study groups					
	GALA, all patients (n = 646)	GALA, Mexican American patients (n = 291) [†]	GALA, Puerto Rican patients (n = 355) [†]	SAGE (n = 264)	SAPPHIRE (n = 430) [‡]	SAPPHIRE group treated with 6 wk of ICS therapy [‡] § (n = 165)
rs881152	.106	.174	.347	.061	.539	.712
rs2070996	.438	.349	.882	—	—	—
rs28372789	.122	.275	.294	—	—	—
rs34507926	.675	.860	.898	.020	.438	.773
rs7702178	.599	.138	.526	—	—	—
rs2431663	.404	.422	.343	—	—	—
rs3805476	.851	.336	.581	—	—	—
rs322351	.144	.283	.365	—	—	—
rs178723	.249	.487	.349	—	—	—
rs881811	.211	.271	.574	—	—	—

**P* values are presented for Hardy-Weinberg equilibrium.

[†]Values are presented for the GALA study cohort stratified by self-reported ethnicity.

[‡]The SAPPHIRE cohort included those who were screened and genotyped (n = 430) and those who were prospectively treated with 6 weeks of ICS therapy as part of the treatment trial (n = 165). In this study, 48 individuals were in both groups. The SAPPHIRE cohort used in this analysis was limited to those who reported being African American.

§Patients were treated with beclomethasone dipropionate HFA twice daily per MDI and assessed for a change in FEV₁ after 6 weeks of therapy.