

Original article

The *PTGDR* gene is not associated with asthma in 3 ethnically diverse populations

Yuhjung J. Tsai, MD,^a Shweta Choudhry, PhD,^a Jennifer Kho, BS,^a Kenneth Beckman, PhD,^b Hui-Ju Tsai, PhD,^a Daniel Navarro, MD,^a Henry Matallana,^a Richard A. Castro, MD,^a Craig M. Lilly, MD,^c Sylvette Nazario, MD,^d Jose R. Rodriguez-Santana, MD,^e Jesus Casal, MD,^d Alfonso Torres, MD,^d Jorge Salas, MD,^f Rocio Chapela, MD,^f H. George Watson, MD,^g Kelley Meade, MD,^b Pedro C. Avila, MD,^{a,h} William Rodriguez-Cintron, MD,^d Michael LeNoir, MD,ⁱ and Esteban González Burchard, MD, MPH,^a on behalf of the Genetics of Asthma in Latino Americans Study and the Study of African Americans, Asthma, Genes and Environments Investigators *San Francisco and Oakland, Calif, Boston, Mass, San Juan, Puerto Rico, Mexico City, Mexico, and Chicago, Ill*

Background: The prostanoid DP receptor (*PTGDR*) gene on chromosome 14q22.1 has been identified as an asthma susceptibility gene. A haplotype with decreased transcription factor binding and transcription efficiency was associated with decreased asthma susceptibility in African American and white subjects.

The significance of *PTGDR* gene variants in asthma has yet to be determined in Latinos, the largest US minority population, nor has the association been replicated in other populations.

Objective: To determine the role of *PTGDR* gene variants in asthma susceptibility and asthma-related traits among the Mexican, Puerto Rican, and African American populations.

Methods: We determined whether single nucleotide polymorphisms (SNPs) and haplotypes in *PTGDR* were associated with asthma and asthma-related traits by family-based and cross-sectional cohort analyses in 336 Puerto Rican

and 273 Mexican asthmatic trios and by case-control analysis among African American subjects with asthma and healthy controls ($n = 352$).

Results: We identified 13 SNPs in the *PTGDR* gene, and 6 were further analyzed. There was no significant association between *PTGDR* variants and asthma by family-based or case-control analyses. SNPs -441C and -197C and haplotype TTT showed marginal association with asthma-related traits in Mexican subjects. SNP -441 genotype TT ($P = .05$) and haplotype TTT ($P = .02$) were associated with increased IgE levels in African Americans.

Conclusion: We conclude that the *PTGDR* gene is not a significant risk factor for asthma among Puerto Ricans, Mexicans, or African Americans.

Clinical implications: Asthma candidate genes provide insights to pathophysiology and potentially new therapeutic targets, although the *PTGDR* gene was not found to be a significant risk factor for asthma in 3 populations. (J Allergy Clin Immunol 2006;117:1016-1022.)

Key words: Asthma genetics, *PTGDR* gene, Latinos, African Americans

From ^athe University of California, San Francisco; ^bthe Children's Hospital and Research Institute, Oakland; ^cBrigham and Women's Hospital, Boston; ^dSan Juan Veterans Affairs Medical Center, University of Puerto Rico School of Medicine; ^ethe Pediatric Pulmonary Program of San Juan; ^fInstituto Nacional de Enfermedades Respiratorias, Mexico City; ^gthe James A. Watson Wellness Center, Oakland; ^hNorthwestern Memorial Hospital, Chicago; and ⁱBay Area Pediatrics, Oakland.

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Reprint requests: Esteban González Burchard, MD, Box 2911, Rock Hall 1550 4th St, SF 584C, University of California, San Francisco, San Francisco, CA 94143-2911. E-mail: Esteban@sfg.ucsf.edu.

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The prostanoid DP receptor (*PTGDR*) on chromosome 14q22.1 has recently been identified by Oguma et al¹ as an asthma susceptibility gene in a functional and case-control analysis of white and African American subjects with asthma. The *PTGDR* gene product is a heptahelical transmembrane G-protein-coupled receptor containing 2 exons and 359 amino acids.² Prostaglandin D₂, the ligand for *PTGDR*, is a mast cell-derived mediator. In turn, *PTGDR* mediates T-cell chemotaxis. In response to antigen challenge with ovalbumin, *PTGDR*^{-/-} mice show a decrease in signs of inflammation, including T_H2 cytokine levels and lymphocyte accumulation in the lungs.³ In addition, *PTGDR*^{-/-} mice do not develop airway hyperactivity and show marginal eosinophil infiltration.

The study by Oguma et al¹ is distinct in providing mechanistic support in an asthma association study. They identified 6 single nucleotide polymorphisms (SNPs) in *PTGDR*, 3 each in the coding and the promoter

Abbreviations used

AIM:	Ancestry informative marker
BMI:	Body mass index
GALA:	Genetics of Asthma in Latino Americans Study
LD:	Linkage disequilibrium
MX:	Mexico City
NY:	New York City
PR:	Puerto Rico
<i>PTGDR</i> :	Prostanoid DP receptor gene
SAGE:	Study of African Americans, Asthma, Genes and Environments
SF:	San Francisco Bay area
SNP:	Single nucleotide polymorphism
TDI:	Transmission disequilibrium test

regions. The SNPs in the coding region result in both conservative (C367A: Leu123Ile) and synonymous (G894A: Arg298Arg, G1044A: Arg348Arg) amino acid changes. Association analyses showed that SNP T-549C in the promoter was associated with asthma in both white and African American populations after adjusting for age and sex. The promoter SNP C-441T was also associated with asthma susceptibility in white subjects.

Functional studies suggested that 3 SNP haplotypes (-549T/C, -441C/T, -197T/C) for the promoter differ in binding to and activation by transcription factors C/EBP β and members of the GATA and Sp group. The TCT promoter haplotype, with lower transcription efficiency, was associated with decreased asthma susceptibility in both African American subjects ($P = .03$) and white subjects ($P = .002$). The CCC haplotype, with high transcription efficiency, was more common among white subjects with asthma than controls; this finding approached marginal statistical significance ($P = .08$).

Latino children make up the largest demographic among all US children.⁴ US vital statistics also suggest that asthma prevalence, morbidity, and mortality are highest in Puerto Ricans and lowest in Mexicans, with a 4-fold difference in asthma burden.⁵⁻⁷ Furthermore, in the United States, age-specific asthma mortality and hospitalization rates are consistently highest among African Americans, while the prevalence of asthma is highest among Puerto Ricans.^{8,9} It remains to be seen whether *PTDGR* will be associated with asthma in Latinos, or whether the previous associations in African Americans can be replicated.

The Genetics of Asthma in Latino Americans Study (GALA) is an ongoing international collaboration to identify novel genetic and clinical risk factors associated with asthma in Mexican and Puerto Ricans, the 2 largest Latino populations in the United States. Given the severity of asthma among African Americans, we have also initiated the Study of African Americans, Asthma, Genes and Environments (SAGE), a case-control study.

To test for associations between *PTGDR* SNPs and haplotypes with asthma and asthma-related phenotypes in the GALA and SAGE populations, we performed

SNP discovery for the gene and genotyped the same 6 SNPs (T-197C, C-441T, T-549C, C+367A, G+894A, and G+ 1044A) described in the original study.¹ We then performed family-based and case-control tests of association in the GALA trios and the SAGE subjects, respectively. The family-based approach is robust to the effects of population stratification, a potentially important confounding factor.¹⁰

METHODS

Study participants

A total of 684 Latino subjects with asthma (probands) and their biological parents were recruited from the San Francisco Bay area (SF), New York City (NY), Puerto Rico (PR), and Mexico City (MX). African American subjects with asthma and healthy controls were enrolled in SF for the SAGE study. Clinical characteristics of Mexican (SF [$n = 218$] and MX [$n = 118$] sites), Puerto Rican (NY [$n = 179$] and PR [$n = 94$] site), and African American subjects with asthma are shown in Table I. The median age of the Mexican, Puerto Rican, and African American subjects with asthma was 13.3, 12.0, and 19.4 years, respectively.

Ethnicity was self-reported. In both GALA and SAGE studies, subjects with asthma were enrolled only if both biological parents and all 4 biological grandparents were of the same ethnic backgrounds: Puerto Rican at NY and PR, Mexican at SF and MX, and African American at SF. Additional inclusion criteria include subject age 8 to 40 years, a current physician diagnosis of asthma, and presence of asthma symptoms (wheezing, cough, or shortness of breath) over the last 2 years. The American Thoracic Society Division of Lung Diseases Epidemiological Questionnaire, modified to assess frequency and duration of asthma and allergy symptoms, was used to assess asthma characteristics.¹¹ Recruitment criteria were identical at each site. Local Institutional Review Boards approved all studies, and all subjects provided written, age-appropriate informed consent or assent.

Pulmonary function tests and IgE measurements

Spirometry was performed according to American Thoracic Society standards.¹² Pulmonary function test results are shown in Table I and are expressed as a percentage of the predicted normal value using age-adjusted prediction equations from Hankinson et al.¹³ A quantitative measure of bronchodilator responsiveness was calculated as ΔFEV_1 , which is relative percent change in pre- FEV_1 (baseline FEV_1 expressed as percent of predicted) after administration of 180 μ g albuterol. Total plasma IgE was measured in duplicate using UniCAP technology (Pharmacia, Kalamazoo, Mich).

SNP discovery

The SNP discovery panel includes 72 unrelated subjects with asthma, 24 each of Mexican, Puerto Rican, and African American ethnicities (144 chromosomes). This number of subjects gives us greater than 90% power to detect variants with an allele frequency of 5% or more. All exons, exon-intron boundaries, and the 1-kb promoter region upstream of the *PTGDR* gene were sequenced. Sequencing was performed using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif) and ABI Prism 3700 sequencer (Applied Biosystems).

Genotyping

All SNPs were genotyped using a fluorogenic allele-specific amplification method (Amplifluor; Chemicon, Temecula, Calif). Allele-specific PCR primers were obtained from Integrated DNA

TABLE I. Characteristics and pulmonary function results of GALA asthmatic probands (Puerto Ricans and Mexicans) and SAGE asthmatic probands (African Americans) included in the *PTGDR* analysis*

Characteristic	Comparison by ethnicity		
	Puerto Ricans (n = 336) 218 from PR; 118 from NY	Mexicans (n = 273) 179 from SF; 94 from MX	African Americans (n = 176)
Characteristic			
Age (y)	12.0 (10:15)	13.3 (11:20)	19.4 (13:31)
Sex (% male)	56.7%	53.0%	35.2 %
BMI (kg/m ²)	21.3 (17:26)	23.7 (20:28)	25.8 (22:32)
Serum IgE (IU/mL)	254 (95:632)	246 (100:591)	164 (48:342)
Baseline spirometry			
Pre-FEV ₁	83 (74:93)	89 (77:100)	93.15 (84:102)
Pre-FEV ₁ < 80%	38%	30%	21%
Bronchodilator responsiveness			
ΔFEV ₁ (relative % predicted)	4.9 (0.6:10)	7.4 (4:13)	7.5 (4:14)

*Values are expressed as median (25th:75th percentile) and were missing for some subjects. Analysis was performed by Mann-Whitney rank test.

Technologies (Coralville, Iowa). PCR reactions (2 μ L final volume) included 5 ng genomic DNA, 25 nmol/L allele-specific forward primers, 375 nmol/L universal forward primers, 375 nmol/L reverse primer, 1x Amplifluor buffer, 1.8 mmol/L MgCl₂, 200 μ mol/L deoxyribonucleotide triphosphate (dNTPs), and 0.05 U/ μ L JumpStart Taq (Sigma, St Louis, Mo). PCR cycling was performed in 384-well format on an MJ Tetrad PTC-225 (Bio-Rad, Hercules, Calif) as follows: 4 minutes $\times$ 96°C to activate the JumpStart Taq polymerase, 20 cycles of 95°C $\times$ 15 seconds, 60°C $\times$ 15 seconds, 72°C $\times$ 60 seconds, followed by 15 to 17 cycles of 95°C $\times$ 15 seconds, 55°C $\times$ 60 seconds, and 72°C $\times$ 60 seconds. Cycling was followed with a 3-minute hold at 72°C. After amplification, fluorescence was read using an ABI 7900HT (Applied Biosystems), and cluster analysis was performed by using SDS v 2.1.1 software (Applied Biosystems). All plates included no-template controls.

Statistical analysis

Hardy-Weinberg equilibrium was calculated by means of χ^2 goodness-of-fit tests. The degree of linkage disequilibrium (LD) was estimated by using the r^2 statistic.¹⁴ In the GALA trios, mendelian inconsistencies were identified using the program PEDCHECK.¹⁵ The transmission disequilibrium test (TDT) was performed among GALA trios to assess the association between *PTGDR* SNPs, genotypes and haplotypes with asthma disease status, and asthma qualitative and quantitative traits. The TDT analysis was performed using the programs FBAT¹⁶ (Boston, Mass) and HaploFBAT¹⁷ (Boston, Mass) assuming an additive model. Quantitative phenotypes included asthma severity as defined by pre-FEV₁, FEV₁/forced vital capacity (FVC), and forced expiratory flow at 25% to 75% of FVC; bronchodilator responsiveness (Δ FEV₁, relative % of predicted); and IgE levels, log₁₀-transformed for normal distribution. Qualitative phenotypes included pre-FEV₁ greater or less than 80%, and Δ FEV₁ greater or less than 12%. The comparison of SNP, genotype, and haplotype frequencies in SAGE African American case and control subjects was made using χ^2 analysis. *PTGDR* haplotypes were determined in the SAGE population by the program PHASE (Seattle, Wash). The association tests between genotypes/haplotypes with asthma and quantitative traits were performed by using a logistic regression model and with quantitative traits by using a linear regression model. All the case-control and cross-sectional analyses were adjusted for potential confounders

such as sex, age, asthma duration, log-transformed body mass index (BMI), steroid use, and regular or as-needed bronchodilator use. The case-control analysis in the African American population may be confounded because of population stratification. To correct for the effect of population stratification in SAGE, all case-control and cross-sectional analyses were also adjusted for individual admixture using 30 unlinked ancestry informative genetic markers (AIMs; see this article's [Methods](#) in the Online Repository at www.jacionline.org). We used STATA 8.0 S/E statistical software (College Station, Tex) to run all multivariate analyses.

RESULTS

SNP discovery, Hardy Weinberg Equilibrium, and LD

Twelve SNPs were identified in the *PTGDR* gene in our SNP discovery panel: 8 in the promoter region, 1 in exon 1, and 3 in exon 2. Only 4 SNPs (-549T/C, -441C/T, -197T/C, 1044G/A) were present at greater than 5% frequency in the combined populations. Six out of 12 SNPs identified were novel (see this article's [Table E1](#) in the Online Repository at www.jacionline.org), although none of the novel SNPs was present in greater than 5% in the aggregate population. The 4 SNPs with greater than 5% frequency, in addition to 2 that were evaluated in the original *PTGDR* study (+367 C/A, +894 G/A) were further genotyped in the entire GALA and SAGE populations. All 6 studied SNPs were in Hardy-Weinberg equilibrium among Mexican and Puerto Rican parents and African American controls, except for SNP -441 in Mexican parents ($P = .04$). Given that 6 SNPs were tested in 3 populations, it is likely that 1 SNP would be out of HWE by chance. Pairwise LD between positions -549/-441 was strongest in Mexicans ($r^2 = 0.58$), followed by African Americans ($r^2 = 0.50$) and Puerto Ricans ($r^2 = 0.44$). There was tight LD ($r^2 > 0.7$) between SNP -197/+1044 in the Mexican GALA samples ($r^2 = 0.73$) and, to a lesser degree, in Puerto Ricans ($r^2 = 0.66$). LD between these 2 SNPs was

TABLE II. *PTGDR* SNP and haplotype frequencies (>1%) in GALA Puerto Rican and Mexican probands and African American SAGE subjects with asthma*

SNP/haplotype	Alleles/ haplotype	Amino acid change	Allele/ haplotype	Frequency (%)				
				Puerto Rican subjects	Mexican subjects	African American subjects	Oguma white subjects	Oguma African American subjects
-549	T/C	—	T	52	69	48	50	46
-441	C/T	—	C	68	42	68.6	73	68
-197	T/C	—	T	88	93	89.7	88	88
(rs11157907)								
+894	G/A	Arg298/ Arg298	G	99	100	99.8	>99	>99
+1044	G/A	Arg348/ Arg348	G	91	94	96	88	91
(rs17125273)								
Haplotype 1	T/T/T	—	1	32	58	31.6	27	33
Haplotype 2	C/C/T	—	2	36	24	41.7	39	43
Haplotype 3	T/C/T	—	3	20	11	16.4	22	13
Haplotype 4	C/C/C	—	4	12	7	10	12	11

*The allele frequencies in subjects with asthma from the study by Oguma et al¹ are listed for comparison. Haplotype SNP order: T-549C, C-441T, T-197C. Reference sequence numbers, when available, are listed.²⁵

low ($r^2 = 0.20$) among African Americans. The LD patterns in 3 populations are available in this article's Fig E1 in the Online Repository at www.jacionline.org.

Allele frequencies

Out of 684 GALA trios and 352 SAGE cases and controls genotyped for 6 *PTGDR* SNPs, we obtained complete genotype information on 609 GALA families: 336 Puerto Rican, 273 Mexican, and 176 SAGE African American subjects with asthma and 176 controls. Table II describes the frequency of the alleles and haplotypes in the GALA and SAGE subjects with asthma compared with those observed in previous studies.^{1,10} The *PTGDR* allele and haplotype frequency in the SAGE subjects were comparable with the African American subjects in the study by Oguma et al.¹ *PTGDR* SNP +367 was monomorphic for the C allele in all our populations, and more than 99% of the subjects had the +894G allele. Therefore, both SNPs were excluded from subsequent analyses. Similarly, both the G894A and C367A alleles were found in less than 1% of the population and were not studied further.

Association analysis of *PTGDR* SNPs with asthma, asthma severity, bronchodilator response, and IgE levels

No individual *PTGDR* SNP was significantly over-transmitted to asthmatic probands ($P > .05$) in the family-based analysis of Puerto Rican and Mexican subjects (Table III). We also evaluated for possible association between *PTGDR* SNPs and quantitative and qualitative traits of asthma. In this analysis, the -441C allele was associated with higher pre-FEV₁, and -197C was associated with decreased bronchodilator drug responsiveness to albuterol, Δ FEV₁ <12%, among Mexicans. However, these associations were weak and did not remain significant after correction for multiple testing. We also performed the same analyses separately for each recruitment

site—NY and PR for Puerto Rican trios and SF and MX for Mexican trios—but observed no additional significant associations between any SNPs and asthma-related traits (data not shown).

In the case-control χ^2 analysis of African American subjects, none of the 4 SNPs tested was associated with a diagnosis of asthma (Table IV). In the genotype analysis, -441TT was associated with increased log IgE ($P = .048$). Because phenotypic heterogeneity may be a cause of different results among association studies, our cross-sectional analyses were adjusted for potential confounders such as sex, age, log BMI, asthma duration, and medication use. We also included individual admixture estimates (determined using 30 unlinked autosomal AIMs; see this article's Table E2 in the Online Repository at www.jacionline.org) to adjust for potential confounding caused by population stratification in cross-sectional analyses of asthma and asthma traits in SAGE subjects. We did not find any potential spurious associations or any additional significant associations between *PTGDR* and asthma or asthma traits after adjusting for individual admixture.

Haplotype-based association analysis

In the previous *PTGDR* analysis,¹ the TCT haplotype at positions -549, -441, and -197 showed decreased transcription factor binding, transcriptional efficiency, and asthma susceptibility, whereas haplotype CCC showed a high transcriptional efficiency and approached statistical significance in increasing asthma susceptibility. In our family-based analysis, neither haplotype was significantly associated with increased or decreased asthma susceptibility. There were no additional associations between haplotypes and asthma in either Puerto Ricans or Mexicans. Neither TCT nor CCC was significantly associated with any asthma-related traits in the Puerto Rican or Mexican populations. The TTT haplotype was associated with pre-FEV₁ in Mexican subjects with asthma ($P = .035$).

TABLE III. Family-based association analysis of qualitative and quantitative asthma traits with *PTGDR* SNPs and haplotypes in Puerto Ricans (PR) and Mexicans (MX)*

	SNP				Haplotype			
	-549 T	-441 (C)	-197 (T)	+1044 (G)	Haplotype 1 T/T/T	Haplotype 2 C/C/T	Haplotype 3 T/C/T	Haplotype 4 C/C/C
	<i>P</i> value							
Asthma								
MX	(-); 0.89	(+); 0.61	(-); 0.06	(-); 0.21	(-); 0.44	(-); 0.18	(+); 0.34	(+); 0.24
PR	(-); 0.78	(+); 0.86	(-); 0.45	(-); 0.56	(+); 0.86	(-); 1.00	(-); 0.79	(-); 1.00
Quantitative traits								
Pre-FEV ₁								
MX	(-); 0.22	(+); 0.05	(-); 0.65	(-); 0.66	(-); 0.04	(+); 0.29	(+); 0.44	(+); 0.99
PR	(+); 0.12	(-); 0.53	(+); 0.94	(-); 1.00	(-); 0.10	(+); 0.41	(+); 0.48	(-); 0.62
ΔFEV ₁ (relative % predicted)								
MX	(+); 0.56	(-); 0.12	(+); 0.23	(+); 0.11	(+); 0.17	(-); 0.61	(-); 0.47	(-); 0.37
PR	(-); 0.78	(+); 0.30	(-); 0.50	(-); 0.17	(-); 0.70	(-); 0.32	(+); 0.43	(+); 0.06
Log ₁₀ IgE level								
MX	(+); 0.40	(-); 0.74	(-); 0.30	(-); 0.12	(+); 0.62	(-); 0.11	(+); 0.67	(+); 0.35
PR	(+); 0.90	(-); 0.25	(+); 0.77	(-); 0.96	(-); 0.84	(+); 0.19	(-); 0.38	(-); 0.17
FEV ₁ /FVC pre (% predicted)								
MX	(+); 0.94	(+); 0.22	(-); 0.48	(-); 0.35	(-); 0.13	(-); 0.63	(+); 0.05	(+); 0.66
PR	(-); 0.67	(-); 0.95	(-); 0.52	(-); 0.46	(-); 0.84	(+); 0.19	(-); 0.38	(-); 0.17
FEF ₂₅₋₇₅								
MX	(+); 0.25	(-); 0.51	(+); 0.25	(+); 0.26	(+); 0.43	(-); 0.59	(+); 0.45	(-); 0.38
PR	(+); 0.72	(+); 0.98	(-); 0.81	(+); 1.00	(-); 0.47	(-); 0.98	(+); 0.53	(+); 0.80
Qualitative traits								
Pre-FEV ₁ <80% predicted								
MX	(+); 0.80	(-); 0.81	(-); 0.14	(-); 0.51	(+); 0.89	(-); 0.12	(+); 0.72	(+); 0.53
PR	(-); 0.19	(+); 1.00	(-); 0.58	(-); 0.87	(+); 0.18	(+); 0.92	(-); 0.15	(+); 0.56
Pre-FEV ₁ >80% predicted								
MX	(-); 0.62	(+); 0.24	(-); 0.19	(-); 0.27	(-); 0.21	(-); 0.64	(+); 0.31	(+); 0.40
PR	(-); 0.94	(+); 0.42	(-); 0.41	(+); 0.52	(-); 0.76	(-); 0.57	(+); 0.54	(-); 0.55
ΔFEV ₁ (relative % predicted) >12%								
MX	(+); 0.47	(-); 0.42	(-); 0.61	(+); 0.80	(+); 0.60	(-); 0.18	(+); 0.71	(+); 0.76
PR	(+); 0.67	(-); 0.57	(-); 0.30	(-); 0.35	(-); 0.29	(+); 0.65	(-); 0.87	(+); 0.20
ΔFEV ₁ (relative % predicted) <12%								
MX	(-); 0.47	(+); 0.14	(-); 0.05	(-); 0.07	(-); 0.15	(-); 0.52	(+); 0.37	(+); 0.24
PR	(-); 0.34	(+); 0.41	(-); 0.69	(-); 0.91	(+); 0.31	(-); 0.62	(-); 0.82	(-); 0.35

FEF₂₅₋₇₅, Forced expiratory flow at 25% to 75% of FVC.*+ Or - represents direction of association. Haplotype SNP order: T-549C, C-441T, T-197C. *P* values <.05 are shown in boldface.

There were no significant associations between *PTGDR* haplotypes and asthma among African Americans while controlling for age, sex, regular bronchodilator use, steroid use, asthma duration, and individual admixture estimates. However, haplotype TTT was associated with increased log IgE ($P = .02$) compared with haplotypes CCT, TCT, and CCC. This is consistent with our genotype analysis in which the -441TT genotype, the haplotype's middle allele, was associated with increased log IgE in African Americans.

DISCUSSION

Using both case-control and family-based tests of association, we failed to find robust associations of individual SNPs or SNP haplotypes in the *PTGDR* gene

with asthma in our analysis of Mexican, Puerto Rican, and African American subjects with asthma. This is in contrast to the results reported by Oguma et al,¹ in which both *PTGDR* SNPs and haplotypes were found to be strongly associated with asthma in a white and African American population. To reproduce the earlier analysis, we analyzed our data in various ways. Not only did we perform a global analysis to test for association using various SNP combinations, we also tested individual haplotypes for overtransmission on the basis of the original report. We found no association with asthma for any of these analyses. Although we did everything possible to make our results directly comparable to those from the previous study, it is possible the difference in our association results is caused by phenotypic and/or genetic heterogeneity or by ethnic-specific differences in gene-environment interactions.

TABLE IV. Comparison of *PTGDR* SNP and haplotype frequencies (>5%) among SAGE African American subjects with asthma and controls*

SNP/haplotype	Alleles/haplotype	Amino acid change	Frequency (%)		P value
			African American controls	African American subjects with asthma	
-549	T/C	—	44.6	48	.34
-441	C/T	—	70.0	68.6	.89
-197	T/C	—	87.6	89.7	.16
+1044	G/A	Arg348/Arg348	95.1	96	.29
Haplotype 1	T/T/T	—	30.1	31.6	.73
Haplotype 2	C/C/T	—	42.8	41.7	.83
Haplotype 3	T/C/T	—	14.8	16.4	.66
Haplotype 4	C/C/C	—	12.3	10	.50

*Haplotype SNP order: T-549C, C-441T, T-197C.

It is unlikely that inadequate sample size precluded the replication of the previously reported association. We had complete genotype data from 609 family trios ($n = 1827$ individuals), which is comparable to the sample size that Oguma et al¹ used for their study (518 white subjects with asthma and 175 controls). In addition, we analyzed African American subjects ($n = 176$) and controls ($n = 176$), as did Oguma et al,¹ who also analyzed African American cases ($n = 80$) and controls ($n = 45$). Furthermore, by using the program QUANTO, which computes sample size and power for gene association studies, we find that our family-based and case-control analyses each have greater than 90% power to detect susceptibility locus with a moderate risk (relative risk = 1.6).¹⁸

However, there might be several other potential reasons why our results differed from those reported previously. First, we studied different populations. We studied Latinos recruited from MX, PR, and the continental United States as well as African Americans recruited from SF. Ethnic-specific differences in environmental or genetic risk factors may account for the observed differences.¹⁹ Such gene-environment interactions may be especially important with polygenic condition such as asthma, in which different alleles of a gene may be associated with susceptibility to or protection from a disease.^{20,21} Another important difference between the study by Oguma et al¹ and ours is the potential for phenotypic heterogeneity. The mean age of our subjects with asthma was 12.0, 13.3, and 19.4 years in Puerto Ricans, Mexicans, and African Americans, respectively. In contrast, in the study by Oguma et al,¹ the mean age was 34 and 33 years for white and African American subjects, respectively. These differences in age may reflect different asthma endophenotypes, the biological markers between genotype and external phenotype that may be under the control of different genetic and environmental regulatory mechanisms. Nevertheless, our results suggest that *PTDGR* is not a major gene for asthma, asthma severity, or bronchodilator responsiveness in Puerto Ricans, Mexicans, or African Americans participating in either the GALA or SAGE study.

It is known that the frequency of SNPs can differ between ethnic groups, and we in fact observed as high as 31% allele frequency differences for these SNPs between

Latino and white populations. Therefore, it is possible that additional ethnic-specific SNPs or multilocus genotypes may differentially contribute to asthma in Mexicans, Puerto Ricans, or African Americans that were not identified in the white ($n = 39$) and African ($n = 11$) groups screened by Oguma et al.¹ Furthermore, the genetic basis of asthma may differ between ethnic groups. Although a particular subset of SNPs identified by Oguma et al¹ may be risk factors for asthma in the populations that they studied, a different subset may increase risk in the populations that we analyzed.

Another potential cause for the differences in results is genetic confounding caused by population stratification, in which differences in allele frequencies between cases and controls are a result of ancestral heterogeneity rather than disease status. Oguma et al¹ genotyped 29 unlinked markers in the white and African American populations and found no evidence of population stratification. In the SAGE analysis, we screened 30 AIMs. The TDT approach, our method for analyzing the GALA subjects, is robust against population stratification.¹⁰ In contrast, association studies that do not account for population stratification may lead to a potential excess of both false-positive and false-negative findings.^{19,22,23} We found no evidence of population stratification in the African American population. However, at present, it is unclear how many and what types of markers are necessary to rule in or rule out the effects of population stratification. As such, it is possible that both studies were underpowered to rule out the effects of population stratification. Defining the parameters that identify and correct for population stratification will be an important step to ensure that all populations equally benefit from genetic association studies.

We did find marginal association between *PTGDR* and asthma-related traits. The -441C allele and the TTT haplotype were associated with pre-FEV₁ in Mexicans. However, these results did not remain significant after correcting for multiple testing and were not previously observed in the study by Oguma et al.¹ Log IgE was not associated with any *PTGDR* gene variants in the study by Oguma et al.¹ In contrast, we identified an association between *PTDGR* SNPs and haplotypes and IgE levels in

African Americans. We observed this association only in a single population, and it will be important to replicate these results in additional populations.

Recently we have found ethnic-specific differences in bronchodilator drug responsiveness to albuterol between Puerto Ricans and Mexicans.²⁴ This observed difference may be a result of different gene-environment interactions between Mexican and Puerto Rican subjects with asthma. Thus, it would not have been surprising to see different results in *PTGDR* association between these 2 groups. However, across all 3 populations, we did not find a consistent significant association between *PTGDR* and asthma. These results are of particular importance for the Puerto Rican and African American populations because of the greater asthma morbidity and mortality in these populations. As prostanoid DP receptor antagonists now enter clinical trials for asthma, it will be important to evaluate the effectiveness of this therapy in these 2 populations given the increased asthma burden. Finally, the work of Oguma et al¹ represents an important contribution to the field of asthma by providing consistent mechanistic support for the association between genetic variants and asthma susceptibility. Although our results do not support those reported by Oguma et al,¹ our respective results underscore the genetic and environmental intricacies of a complex disease such as asthma.

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