

CD14 Tobacco Gene–Environment Interaction Modifies Asthma Severity and Immunoglobulin E Levels in Latinos with Asthma

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Background: A recent family-based genomewide screen revealed linkage between the 5q31 region and the diagnosis of asthma, but only in those exposed to environmental tobacco smoke (ETS). Among the candidate genes in this region is CD14. **Methods:** To determine whether polymorphisms in the CD14 gene are related to this gene-by-environment interaction in Latinos, we used both family-based and cross-sectional cohort analysis to test for interactions between CD14 genotypes/haplotypes, exposure to ETS, and asthma-related phenotypes in 659 Mexican and Puerto Rican families. **Results:** We identified 21 single nucleotide polymorphisms (SNPs) in the CD14 gene by sequencing 72 Puerto Ricans, Mexicans, and African Americans with asthma. Three SNPs, –810, –159, and +1437, were further genotyped in families with asthma. Among all subjects with asthma exposed to ETS, without regard to ethnicity, CD14 +1437 genotypes were associated with asthma severity. SNP +1437 GG or GC genotypes were significantly associated with lower baseline FEV₁ using both family-based ($p = 0.0009$) and cross-sectional cohort ($p = 0.03$) analyses. Subjects with asthma with the GG or GC genotypes who were exposed to ETS had mean baseline FEV₁ (% predicted) values 8.6% lower than subjects not exposed to ETS ($p = 0.03$). As previously observed in whites, we found an interaction between plasma IgE levels, SNP –159 genotypes, and ETS exposure ($p = 0.0002$). The lowest IgE levels were in those subjects with the TT genotype and who were exposed to ETS regardless of ethnicity. **Conclusions:** Our data suggest a gene-by-environment interaction between CD14 genotypes and ETS, which affects pulmonary function and IgE levels among Latinos with asthma.

Keywords: asthma genetics; CD14; gene–environment interaction; IgE; Latinos; tobacco

In the United States, Latinos are the largest minority population, and among all children in the United States, Latinos represent the largest demographic group (1). Although both Puerto Ricans and Mexicans are considered Latinos, among Puerto Ricans the asthma morbidity and mortality are fourfold higher than they are in Mexicans (2, 3). Despite these dramatic differences in asthma morbidity and mortality, very little is known about the genetic and environmental factors that contribute to asthma morbidity and mortality in these populations.

A number of different investigative groups have used genetic linkage techniques to identify human chromosome 5q31 as a region likely to contain genes related to asthma and asthma-related phenotypes (4, 5). Recently, it has been demonstrated that the evidence for linkage to asthma susceptibility may be increased when genomewide linkage analyses are stratified based on environmental factors (6). Colilla and others (6) demonstrated a significant increase in logarithm of the odds score between asthma and a 135-cM region that contains 5q31 when affected individuals participating in a genomewide linkage analysis were stratified by environmental tobacco smoke (ETS) exposure, as determined by questionnaire. Among the genes in this region is the CD14 gene, which may play a significant role in modulating total plasma IgE levels and may have age-dependent effects on asthma-related phenotypes, including atopy and airway hyperresponsiveness (7, 8). The CD14 protein is part of the receptor complex for endotoxin (9). Endotoxin is found in cigarette smoke particles (10, 11). Exposure to ETS results in the inhalation of endotoxin levels that are 120 times higher than exposure to smoke-free air (10). Endotoxin is a potent inflammatory agent and may contribute to the high prevalence of respiratory disorders among smokers and among nonsmokers exposed to ETS (10, 11). Genetic variation in the CD14 gene may modify the interaction between ETS, endotoxin exposure, allergic diseases, and asthma severity (12).

A polymorphism in the CD14 gene promoter (C-159T) has been shown to be associated with decreased total plasma IgE levels in white and Chinese children (13, 14). However, results from cross-sectional and case-control studies relating this CD14 gene polymorphism to asthma and IgE levels in different populations have been inconsistent (8, 13–18). Inconsistencies may reflect spurious associations caused by population stratification, random error, and racial/ethnic heterogeneity, which can influence genotypic relative risk (19). Inconsistent results may also

(Received in original form September 17, 2004; accepted in final form April 21, 2005)

Supported by the National Institutes of Health (K23 HL04464, HL07185, GM61390, NCMHD Health Disparities Scholar, American Lung Association of California, Extramural Clinical Research Loan Repayment Program for Individuals from Disadvantaged Backgrounds, 2001–2003, to E.G.B.; HL51823, HL074204, 3M01RR000083-38S30488, HL56443, and HL51831 to the Asthma Clinical Research Network), SFGH General Clinical Research Center (M01RR00083-41, U01-HL 65899), UCSF–Children's Hospital of Oakland Pediatric Clinical Research Center (M01 RR01271), Oakland, CA, Sandler Center for Basic Research in Asthma, and the Sandler Family Foundation.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 172, pp 173–182, 2005

Originally Published in Press as DOI: 10.1164/rccm.200409-1232OC on May 5, 2005

Internet address: www.atsjournals.org

be explained by the difference in environmental factors across studies, which may be important in modifying genetic associations.

We used family-based and cross-sectional cohort analyses and incorporated data on ETS exposure to determine whether C-810A, C-159T, and G+1437C polymorphisms in the CD14 gene are associated with asthma, asthma severity, bronchodilator responsiveness, and IgE levels in Mexican and Puerto Rican subjects with asthma participating in the Genetics of Asthma in Latino Americans (GALA) study. The GALA study is an ongoing, multicenter, international, collaborative effort to identify novel clinical and genetic risk factors associated with asthma and asthma severity among Mexicans and Puerto Ricans, the two largest Latino populations in the United States. Unlike case-control or cross-sectional genetic association studies, family-based analyses are robust against the effects of population stratification (20, 21). We also performed cross-sectional cohort analyses and adjusted for potential confounding factors.

METHODS

Study Participants

Recruitment and patient characteristics are described in detail elsewhere (22). Briefly, 684 subjects with asthma (probands) and their biological parents were enrolled in the San Francisco Bay Area, New York City, Puerto Rico, and Mexico City. Probands were assessed using a modified version of the 1978 American Thoracic Society (ATS)–Division of Lung Disease Epidemiology Questionnaire (23). Ethnicity was self-reported. Probands were enrolled only if both biological parents and all biological grandparents were of Puerto Rican (for those recruited in New York City and San Juan, Puerto Rico) or Mexican ethnicity (from San Francisco and Mexico City sites). Recruitment criteria were identical at each site. Local institutional review boards approved all studies, and all subjects provided written, age-appropriate informed consent.

Asthma and Medical Questionnaire

A trained interviewer administered a modified version of the 1978 ATS questionnaire (23), which was modified to assess the frequency and duration of asthma and allergy symptoms as well as ETS exposure. Questionnaires were available in Spanish and English. ETS exposure was determined to be present if one or both parents or any other person in the household reported smoking during the first 2 years of life in the child with asthma and absent if the answer was “no.” History of

ETS exposure was only collected on children younger than 13 years (139 Mexican and 208 Puerto Rican probands).

Pulmonary Function Tests and IgE Measurements

Pulmonary function tests are described in detail in an online supplement. Spirometry was performed according to ATS standards (24). Pulmonary function test results are shown in Table 1 and are expressed as a percentage of the predicted normal value using age-adjusted prediction equations from Hankinson (25). Total plasma IgE was measured in duplicate using Uni-Cap technology (Pharmacia, Kalamazoo, MI).

Single Nucleotide Polymorphism Discovery

The single nucleotide polymorphism (SNP) discovery in the CD14 gene was performed using the GALA SNP Discovery Panel, which consists of 72 unrelated subjects with asthma: 24 Mexicans, 24 Puerto Ricans, and 24 African Americans. All exons, exon–intron boundaries, and the 2-kb promoter region upstream of the CD14 gene were sequenced. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and ABI Prism 3700 sequencer (Applied Biosystems). The degree of linkage disequilibrium was estimated by using the r^2 statistic (26). An r^2 statistic of 1 implies complete linkage disequilibrium and an r^2 statistic of 0 implies no linkage disequilibrium.

Genotyping

The C to T polymorphism, 159 bp upstream of the transcription start site of the CD14 gene, was detected by the loss of a restriction site for the AVAII restriction enzyme (13). CD14 C-159T genotyping was performed with minor modifications of the methods reported by Baldini and colleagues (13). Polymerase chain reaction primers were obtained from Research Genetics, Inc. (Huntsville, AL), and AVAII enzyme was obtained from New England Biolabs (Beverly, MA). SNPs –810 and +1437 were genotyped using the AcycloPrime-FP SNP detection kit (PerkinElmer) (27). The polymerase chain reaction cocktail included the following: 2.4 to 4.0 ng genomic DNA, 0.1 to 0.2 μ M primers, 2.5 mM MgCl₂, 50 μ M deoxynucleotide triphosphates, 6- μ l volume with Platinum Taq polymerase chain reaction buffer, and 0.1 to 0.2 units Platinum Taq (Invitrogen, Carlsbad, CA) plus 1 μ l extra water to counteract evaporation. Polymerase chain reaction cycling conditions were as follows: 95°C for 2 minutes, 35 cycles of 92°C for 10 seconds, 58°C for 20 seconds, 68°C for 30 seconds, and final extension at 68°C for 10 minutes. We used AcycloPrime-FP kits for enzymatic cleanup and single base extension genotyping reactions. Plates were read on an EnVision fluorescence polarization plate reader.

TABLE 1. CLINICAL CHARACTERISTICS AND PULMONARY FUNCTION RESULTS OF PROBANDS WITH ASTHMA INCLUDED IN THE CD14 ANALYSIS IN THE GENETICS OF ASTHMA IN LATINO AMERICANS STUDY

Characteristic	Comparison by Ethnicity		p Value
	Puerto Ricans (n = 365)	Mexicans (n = 294)	
Age, yr	12.0 (10–15)	13.1 (11–19)	< 0.001
Sex, % male	55.9	54.1	0.66
BMI, kg/m ²	21.2 (17–26)	23.8 (20–28)	< 0.0002
Plasma IgE, IU/ml	258.5 (92–628)	270.0 (99–615)	0.92
Moderate–severe asthma, %	67.1	66.7	0.90
ETS exposure, %	40.97	40.8	0.58
Baseline spirometry			
Pre-FEV ₁ , % predicted	83 (74–93)	90 (77–100)	< 0.0001
Bronchodilator responsiveness			
Δ FEV ₁ , relative % change	5.0 (0.6–10)	7.4 (4–12)	< 0.0001

Definition of abbreviations: BMI = body mass index; ETS = environmental tobacco smoke.

Values are expressed as median (25th–75th percentile), and were missing for some subjects. Analysis was done by Mann-Whitney rank test. ETS exposure was only ascertained in probands younger than 13 years (n = 208 and 139 for Puerto Ricans and Mexicans, respectively).

Statistical Analysis

Mendelian inconsistencies were identified using PedCheck (<http://watson.hgen.pitt.edu/register/docs/pedcheck.html>) (28). Families with Mendelian inconsistencies ($n = 10$) were excluded from further analysis, including Hardy-Weinberg equilibrium and family-based association tests. Hardy-Weinberg equilibrium was calculated by means of χ^2 goodness-of-fit tests.

Family-based association test (FBAT) (29) and HaploFBAT (30) were used to assess associations between the CD14 genotypes and haplotypes with asthma and asthma-related quantitative phenotypes (pre-FEV₁, Δ FEV₁, and IgE levels). IgE values were log₁₀-transformed to become normally distributed. Both additive and dominant models were used for FBAT analysis because dominant model was suggested for SNP -159 in the original report of CD14 (13). Analysis was also performed by stratifying patients on the basis of the following factors: ETS exposure; age (< 12 or > 12 years); breastfeeding; allergies to mold, pollens, or house dust mites; exposure to cats and dogs; and exposure to other large animals.

We used linear regression models (using probands with asthma only) to test for an association between CD14 genotypes and pre-FEV₁ and IgE levels. A dominant model was used for this analysis with dominant allele C, and C and G for SNPs -810, -159, +1437, respectively. Age and sex were entered into the model as covariates. To adjust for potential environmental interactions, second-hand exposure to ETS, birthplace, and exposure to cats, dogs, and other animal pets were also incorporated into models as covariates. Regression analysis was also performed by stratifying patients on the basis of ETS exposure. For selected analyses, an interaction term between genotype and ETS was included in the model. This interaction term was considered to measure the effect of ETS and genotype on asthma severity and IgE levels. All cross-sectional cohort analyses were performed using Stata 8.0 S/E statistical software (Stata Corp., College Station, TX).

RESULTS

Clinical Characteristics, ETS Exposure, and IgE

Clinical characteristics of all probands with asthma are shown in Table 1. Of 684, we analyzed 659 probands, who had a physician diagnosis of asthma and were either currently taking asthma medications or had at least two asthma symptoms (among wheezing, coughing, and shortness of breath) in the previous 2 years. Between Mexican and Puerto Rican probands with asthma, there were no significant differences in total IgE levels (462.5 vs. 491.8 IU/ml, respectively) or ETS exposure rates (40.8 vs. 40.9%, re-

spectively). Total IgE levels did not significantly differ among the entire group of subjects with asthma exposed and not exposed to ETS (data not shown).

SNP Discovery and Linkage Disequilibrium Analysis

A total of 21 SNPs were identified in the CD14 promoter and exonic regions in the GALA SNP Discovery Panel, which consisted of 24 Mexican, 24 Puerto Rican, and 24 African American subjects with asthma (Table 2). Of these 21 SNPs, 17 were found in the promoter region, one in the 5' untranslated region, two in exon 2, and one in the 3' untranslated region. None of the SNPs resulted in an amino acid change. Eleven SNPs had a frequency of more than 10% in one, two, or all three populations. Of these 11 SNPs, three SNPs (-810, -159, and +1437) were selected for further genotyping in the entire set of GALA trios based on the linkage disequilibrium patterns in Puerto Ricans and Mexicans (Table 3).

Allele Frequencies and Hardy-Weinberg Equilibrium

Of the 659 GALA trios, we obtained complete genotyping information for SNPs -810, -159, and +1437 for 621 family trios (362 Puerto Rican and 259 Mexican), which were used for these analyses. The frequencies of SNP -810, -159, and +1437 alleles and genotypes in the combined population (Puerto Rican and Mexican together), Puerto Rican and Mexican probands, and parents are listed in Table 4. The observed distribution of genotypes within each ethnic group was consistent with Hardy-Weinberg equilibrium.

Association Analysis of CD14 SNPs with Asthma, Asthma Severity, Bronchodilator Response, and IgE Levels

Family-based analyses were first performed without stratifying by ETS exposure. We did not observe any significant associations between SNP -810, -159, and +1437 genotypes and asthma, bronchodilator response, or IgE levels among the Mexican, Puerto Rican, or combined populations using either an additive (Table 5) or dominant model (Table 6). However, we observed a significant association between SNP -810 ($p = 0.007$) and

TABLE 2. CD14 SINGLE NUCLEOTIDE POLYMORPHISM (SNP) DISCOVERY USING THE GENETICS OF ASTHMA IN LATINO AMERICANS STUDY SNP DISCOVERY PANEL

Base Pair Position	Polymorphism	Genomic Location	Frequency		
			Puerto Rican	Mexican	African American
-1856	A/G	Promoter	0.64	0.74	0.83
-1672	C/T	Promoter	1.0	1.0	0.95
-1671	G/A	Promoter	1.0	0.98	1.0
-1620	A/G	Promoter	0.74	0.62	0.81
-1280	T/C	Promoter	0.97	1.0	1.0
-1146	A/G	Promoter	0.63	0.59	0.71
-1126	T/G	Promoter	1.0	1.0	0.91
-810	A/C	Promoter	0.63	0.70	0.82
-674	C/G	Promoter	1.0	1.0	0.89
-550	C/T	Promoter	0.62	0.73	0.94
-461	C/T	Promoter	0.74	0.83	0.60
-460	T/A	Promoter	1.0	0.98	0.85
-375	A/T	Promoter	1.0	0.98	0.98
-174	T/G	Promoter	0.98	1.0	1.0
-159	C/T	Promoter	0.67	0.56	0.74
-64	G/A	Promoter	0.98	1.0	1.0
-34	A/G	Promoter	1.0	1.0	0.92
+91	C/T	5' UTR	1.0	1.0	0.97
+261	T/C	Exon2	0.98	1.0	1.0
+1290	G/C	Exon2	0.88	0.90	0.94
+1437	C/G	3' UTR	0.92	0.81	1.0

The SNP Discovery Panel consists of 24 Mexican, 24 Puerto Rican, and 24 African American subjects with asthma. UTR = untranslated region.

TABLE 3. PAIRWISE LINKAGE DISEQUILIBRIUM (r^2) BETWEEN CD14 SINGLE NUCLEOTIDE POLYMORPHISMS WITH MINOR ALLELE FREQUENCY GREATER THAN 10% IN PUERTO RICAN, MEXICAN, AND AFRICAN AMERICAN SUBJECTS WITH ASTHMA

SNP	-1856	-1620	-1146	-810	-674	-550	-461	-460	-159	+1290	+1437
Puerto Rican											
-1856	—										
-1620	0.20	—									
-1146	0.34	0.70	—								
-810	1.00	0.24	0.34	—							
-674	0.00	0.00	0.00	0.00	—						
-550	0.74	0.20	0.34	0.72	0.00	—					
-461	0.24	0.00	0.10	0.26	0.00	0.13	—				
-460	0.05	0.09	0.05	0.05	0.00	0.05	0.07	—			
-159	0.31	0.64	1.00	0.34	0.00	0.30	0.06	0.05	—		
+1290	0.08	0.05	0.09	0.09	0.00	0.08	0.02	0.00	0.07	—	
+1437	0.19	0.04	0.07	0.20	0.00	0.01	0.02	0.00	0.05	0.00	—
Mexican											
-1856	—										
-1620	0.22	—									
-1146	0.27	0.82	—								
-810	0.89	0.25	0.18	—							
-674	0.00	0.00	0.00	0.00	—						
-550	1.00	0.21	0.27	0.89	—						
-461	0.07	0.15	0.14	0.09	0.00	0.08	—				
-460	0.01	0.05	0.04	0.01	0.00	0.01	0.11	—			
-159	0.27	0.82	1.00	0.18	0.00	0.29	0.15	—			
+1290	0.05	0.08	0.09	0.05	0.00	0.04	0.03	0.00	0.09	—	
+1437	0.57	0.12	0.17	0.56	0.00	0.69	0.05	0.01	0.18	0.03	—
African American											
-1856	—										
-1620	0.05	—									
-1146	0.08	0.75	—								
-810	1	0.05	0.09	—							
-674	0.03	0.03	0.04	0.02	—						
-550	0.64	0.02	0.03	0.64	0.01	—					
-461	0.02	0.14	0.25	0.02	0.12	0.00	—				
-460	0.03	0.04	0.08	0.03	0.00	0.02	0.27	—			
-159	0.07	0.66	0.89	0.08	0.00	0.02	0.19	0.05	—		
+1290	0.02	0.01	0.01	0.00	0.01	0.00	0.05	0.00	0.02	—	
+1437	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	—

Definition of abbreviation: SNP = single nucleotide polymorphism.

+1437 ($p = 0.03$) and asthma severity as defined by pre-FEV₁ among Mexican asthmatic trios (Table 5). The association between SNP +1437 and pre-FEV₁ became stronger when both Mexican and Puerto Rican trios were analyzed together ($p = 0.01$). There were no significant associations between CD14 genotypes and asthma-related traits when analyses were stratified by age, breastfeeding, allergies to mold, house dust mites, or pollens, and exposure to cats and dogs or other large animals (data not shown).

Genotype interaction with tobacco exposure. To test the possibility of an interaction between CD14 genotypes and ETS and its effect on asthma and asthma-related phenotypes, we stratified families according to whether the proband had been exposed to ETS. Among Mexican probands exposed to ETS, there was a significant association between SNP -159 and +1437 genotypes and pre-FEV₁, a quantitative measure of asthma severity ($p = 0.002$ and 0.007 , respectively; Table 6). However, among Puerto Ricans, we only observed significant association for asthma severity with SNP +1437 ($p = 0.04$). The association between SNP +1437 and pre-FEV₁ became more significant for the combined population ($p = 0.0009$) with GG/GC genotypes associated with lower baseline FEV₁ among probands exposed to ETS (Table 6). The haplotype analysis using all three SNPs also showed that the haplotype carrying the G allele at position +1437 was associated with lower pre-FEV₁ among combined subjects ex-

posed to ETS ($p = 0.002$; Table 7). Because family-based analyses suggested an association between SNP +1437 genotypes, ETS, and pre-FEV₁, we tested the clinical magnitude of the effect of SNP +1437 genotypes and ETS on pre-FEV₁ levels by cross-sectional analysis in Mexican and Puerto Rican probands. Although these results may be subject to confounding because they are based on comparison of unrelated individuals, results for this analysis were consistent with the family-based associations in the Mexican and the Puerto Rican populations. There was a significant association when subjects were stratified by genotype and ETS exposure. Subject with asthmatics, Puerto Rican and Mexican combined, who had the GG or GC genotype and who were exposed to ETS had a mean pre-FEV₁ value of $78.5 \pm 3.2\%$, which was 8.6% lower than subjects with the GG or GC genotype and who were not exposed to ETS ($87.1 \pm 1.7\%$; Figure 1; $p = 0.03$). There was no significant difference in pre-FEV₁ levels by ETS exposure in the group with CC genotype ($p = 0.53$). Taken together, these results demonstrate an interaction between exposure to ETS and genetic variants in the CD14 gene that is related to asthma severity. In the family-based analysis for Δ FEV₁ stratified by ETS, we observed a significant association with SNP +1437 in Mexican trios ($p = 0.01$) and a marginal association for combined population ($p = 0.09$; Table 6). GG or GC genotype at SNP +1437 was associated with better drug response. We did not find any significant association between CD14

TABLE 4. ALLELE AND GENOTYPE FREQUENCIES AND *p* VALUES FOR HARDY-WEINBERG EQUILIBRIUM ANALYSIS FOR CD14 SINGLE NUCLEOTIDE POLYMORPHISM –810, –159, AND +1437 IN PUERTO RICAN, MEXICAN, AND COMBINED PUERTO RICAN AND MEXICAN PROBANDS AND PARENTS IN THE GENETICS OF ASTHMA IN LATINO AMERICANS STUDY

Allele	Frequency						Leung Chinese	Baldini Whites
	Combined Probands, PR + Mex	PR Probands	Mex Probands	Combined Parents, PR + Mex	PR Parents	Mex Parents		
SNP −810								
C	0.24	0.26	0.22	0.24	0.27	0.21		
A	0.76	0.73	0.78	0.76	0.73	0.79		
Genotype								
CC	0.06	0.07	0.04	0.06	0.08	0.04		
CA	0.38	0.39	0.35	0.37	0.38	0.35		
AA	0.57	0.54	0.61	0.57	0.55	0.61		
HWE (p value)	0.65	0.85	0.52	0.71	0.40	0.44		
SNP −159								
C	0.51	0.54	0.46	0.52	0.55	0.47	0.44	0.51
T	0.49	0.46	0.54	0.48	0.45	0.53	0.56	0.49
Genotype								
CC	0.25	0.29	0.19	0.26	0.30	0.20	0.31	0.24
CT	0.51	0.50	0.54	0.52	0.51	0.53	0.50	0.50
TT	0.24	0.21	0.27	0.22	0.19	0.27	0.19	0.26
HWE (p value)	0.57	0.93	0.22	0.16	0.35	0.14		
SNP +1437								
G	0.08	0.06	0.12	0.08	0.06	0.11		
C	0.92	0.95	0.88	0.92	0.94	0.86		
Genotype								
GG	0.01	0.00	0.02	0.01	0.01	0.01		
GC	0.15	0.11	0.20	0.14	0.10	0.20		
CC	0.85	0.89	0.78	0.85	0.89	0.79		
HWE (p value)	0.63	0.27	0.41	0.55	0.57	0.90		

Definition of abbreviations: HWE = Hardy-Weinberg equilibrium; Mex = Mexican; PR = Puerto Rican; SNP = single nucleotide polymorphism.

The allele frequencies for SNP –159 from the studies reported by Leung and colleagues (14) and Baldini and coworkers (13) are listed for comparison.

genotypes and asthma when stratified by ETS exposure among Mexican, Puerto Rican, or a combined population (Table 6).

Gene-by-Environment Interaction and IgE Levels

When family-based analyses were stratified by ETS, there were significant associations between the CD14 SNP –159 genotypes

and IgE levels among Mexican, Puerto Rican, and the combined populations ($p = 0.006, 0.04, \text{ and } 0.001$, respectively; Table 6). In addition to SNP –159, a marginal association was observed between SNP –810 and IgE levels in combined populations ($p = 0.05$; Table 6). Because family-based analyses suggested an association between SNP –159 genotypes and IgE levels in the

TABLE 5. FAMILY-BASED ASSOCIATION ANALYSIS (*p* VALUES) OF CD14 –810, –159, AND +1437 SINGLE NUCLEOTIDE POLYMORPHISMS AND ASTHMA, ASTHMA SEVERITY, BRONCHODILATOR RESPONSE, AND IgE LEVELS FOR COMBINED PUERTO RICAN/MEXICAN, PUERTO RICAN, AND MEXICAN ASTHMA TRIOS

Phenotype	PR + Mex (<i>n</i> = 621)			PR (<i>n</i> = 362)			Mex (<i>n</i> = 259)		
	–810 C/A	–159 C/T	+1437 G/C	–810 C/A	–159 C/T	+1437 G/C	–810 C/A	–159 C/T	+1437 G/C
Asthma									
Asthma	0.96	0.51	0.81	0.85	0.49	0.38	0.87	0.85	0.67
Exposed to ETS	0.62	0.66	0.62	0.45	0.19	0.23	0.87	0.31	0.66
Not exposed to ETS	0.53	0.28	0.87	0.82	0.19	0.17	0.46	0.91	0.24
Asthma severity									
Pre-FEV ₁ , % predicted	0.09	0.35	0.01*	0.85	0.94	0.24	0.007*	0.15	0.03*
Exposed to ETS	0.44	0.34	0.0008*	0.58	0.59	0.04*	0.05	0.02*	0.006*
Not exposed to ETS	0.35	0.23	0.61	0.53	0.51	0.93	0.06	0.31	0.51
Bronchodilator responsiveness									
ΔFEV ₁ , relative % change	0.91	0.74	0.27	0.16	0.57	0.75	0.08	0.23	0.15
Exposed to ETS	0.84	0.65	0.09	0.02*	0.63	0.57	0.11	0.25	0.02*
Not exposed to ETS	0.92	0.28	0.99	0.27	0.07	0.58	0.13	0.27	0.71
Plasma IgE, log of IU/ml									
Log ₁₀ IgE	0.29	0.15	0.87	0.78	0.82	0.54	0.17	0.06	0.78
Exposed to ETS	0.05	0.02*	0.94	0.18	0.20	0.67	0.12	0.03*	0.73
Not exposed to ETS	0.81	0.96	0.20	0.58	0.41	0.75	0.19	0.35	0.20

Definition of abbreviations: ETS = environmental tobacco smoke; Mex = Mexican; PR = Puerto Rican.

Note that *n* refers to the number of trios analyzed.

* *p* value < 0.05.

TABLE 6. FAMILY-BASED ASSOCIATION ANALYSIS (p VALUES) OF CD14 SINGLE NUCLEOTIDE POLYMORPHISM -810, -159, AND +1437 GENOTYPES AND ASTHMA, ASTHMA SEVERITY, BRONCHODILATOR RESPONSE, AND IgE LEVELS FOR COMBINED PUERTO RICAN/MEXICAN, PUERTO RICAN, AND MEXICAN ASTHMA TRIOS

Phenotype	PR + Mex (n = 621)			PR (n = 362)			Mex (n = 259)		
	-810 CC+CA	-159 CC+CT	+1437 GG+GC	-810 CC+CA	-159 CC+CT	+1437 GG+GC	-810 CC+CA	-159 CC+CT	+1437 GG+GC
Asthma									
Asthma	0.68	0.66	0.69	0.73	0.20	0.48	0.81	0.45	0.91
Exposed to ETS	0.77	0.89	0.27	0.71	0.99	0.17	0.99	0.83	0.89
Not exposed to ETS	0.53	0.23	0.81	0.79	0.17	0.16	0.50	0.74	0.47
Asthma severity									
Pre-FEV ₁ , % predicted	0.05	0.45	0.02*	0.85	0.29	0.22	0.008*	0.03*	0.05
Exposed to ETS	0.37	0.53	0.0009*	0.75	0.05	0.04*	0.08	0.002*	0.007*
Not exposed to ETS	0.13	0.07	0.46	0.95	0.37	0.93	0.04*	0.11	0.36
Bronchodilator responsiveness									
ΔFEV ₁ , relative % change	0.70	0.35	0.42	0.41	0.67	0.80	0.12	0.07	0.10
Exposed to ETS	0.76	0.90	0.09	0.26	0.44	0.57	0.24	0.47	0.01*
Not exposed to ETS	0.94	0.63	0.43	0.32	0.06	0.58	0.15	0.15	0.50
Plasma IgE, log of IU/ml									
Log ₁₀ IgE	0.19	0.14	0.90	0.57	0.61	0.60	0.17	0.11	0.54
Exposed to ETS	0.05*	0.001*	0.98	0.15	0.04*	0.79	0.18	0.006*	0.74
Not exposed to ETS	0.67	0.87	0.25	0.83	0.62	0.75	0.32	0.51	0.25

For definition of abbreviations, see Table 5.

Analyses were performed using a dominant model and stratified by exposure to ETS; n refers to the number of trios analyzed.

* p value < 0.05.

presence of ETS, we performed a cross-sectional cohort analysis among the combined population to test for association between IgE levels and the interaction term (genotype * ETS) to determine if there was evidence of a gene-by-environment interaction. Among the combined population, there was a statistically significant interaction between SNP -159 genotypes and ETS exposure ($p = 0.005$). However, when Mexican and Puerto Rican populations were analyzed separately, this interaction was only significant among the Puerto Ricans ($p = 0.003$). Because there was evidence of a gene-by-environment interaction, we then tested the clinical magnitude of the effect of the SNP -159

genotypes on IgE levels by cross-sectional analysis in Mexicans and Puerto Ricans separately. Among probands exposed to ETS, we found a significant association between SNP -159 genotypes and IgE levels in the Puerto Rican and the combined population ($p = 0.00008$ and 0.0002 , respectively) and a marginal association in the Mexican population ($p = 0.04$). Log₁₀ IgE levels separated by genotype and ETS exposure are depicted in Figure 2. The probands with TT genotypes and exposed to ETS had significantly lower IgE levels compared with probands with the CC or CT genotypes. There was no significant difference in IgE levels by genotype in the group not exposed to ETS.

TABLE 7. FAMILY-BASED ASSOCIATION ANALYSIS (p VALUES) OF CD14 -810/-159/+1437 HAPLOTYPES AND ASTHMA, ASTHMA SEVERITY, BRONCHODILATOR RESPONSE, AND IgE LEVELS FOR COMBINED PUERTO RICAN/MEXICAN, PUERTO RICAN, AND MEXICAN ASTHMA TRIOS

Phenotype	PR + Mex (n = 621)				PR (n = 362)				Mex (n = 259)			
	ATC	ACC	CCC	CCG	ATC	ACC	CCC	CCG	ATC	ACC	CCC	CCG
Asthma	47.8%	27.2%	16.9%	7.1%	44.4%	29.1%	20.7%	5.1%	52.9%	24.5%	11.1%	10.2%
Asthma												
Exposed to ETS												
Not exposed to ETS												
Asthma severity												
Pre-FEV ₁ , % predicted				0.004				0.01				
Exposed to ETS				0.002				0.04	0.04			0.02
Not exposed to ETS												
Bronchodilator responsiveness												
ΔFEV ₁ , relative % change												
Exposed to ETS							0.04*					0.03*
Not exposed to ETS												
Plasma IgE, log of IU/ml												
Log ₁₀ IgE												
Exposed to ETS	0.04		0.05*		0.09				0.08			
Not exposed to ETS												

For definition of abbreviations, see Table 5.

Note that n refers to the number of trios analyzed; p values > 0.1 are not shown in the table.

* For quantitative traits, p values represent haplotypes associated with higher value of the trait.

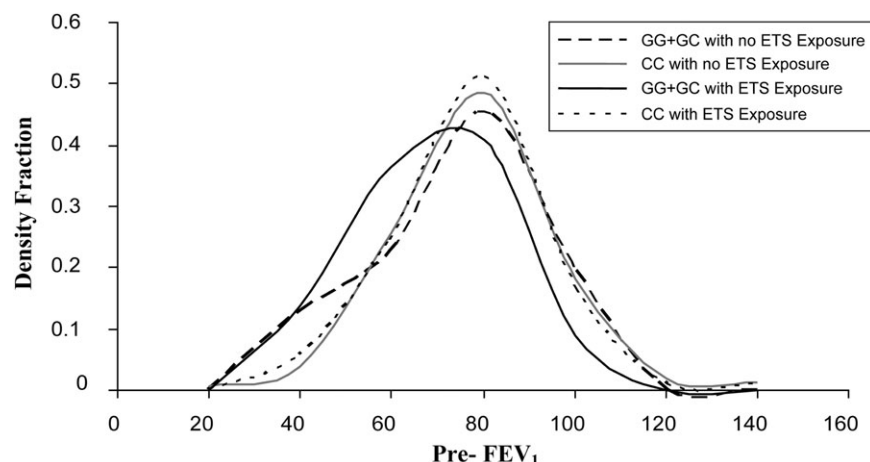


Figure 1. Population density plot for deciles of pre-FEV₁ in combined (Mexican + Puerto Rican) subjects with asthma stratified by single nucleotide polymorphism (SNP) +1437 genotype and environmental tobacco smoke (ETS) exposure. Note that the subjects with asthma with GG or GC genotype that were exposed to ETS have lower pre-FEV₁ than the group not exposed to ETS.

DISCUSSION

We have demonstrated gene-by-environment interactions between CD14 genotypes and asthma severity (pre-FEV₁) and IgE levels in the presence of ETS among Mexicans and Puerto Ricans with asthma. Among Mexicans and Puerto Ricans with asthma exposed to ETS, SNP +1437 GG or GC genotypes were significantly associated with lower FEV₁, both using family-based and cross-sectional analyses. In addition, as previously observed in whites, we also found in Latinos an interaction among plasma IgE levels, SNP -159 genotype, and ETS exposure.

These data suggest a complex relationship between CD14 genotypes, IgE levels, and asthma severity. One of these factors is a gene-by-environment interaction between the SNP +1437 and SNP -159 and ETS, which results in a significant decrement in pulmonary function and lower IgE levels among Mexicans and Puerto Ricans with asthma. This environmental toxicogenetic association has important clinical and public health implications because Latino ethnic groups carry a disproportionate burden

of asthma and Puerto Ricans have the highest rates of smoking among Latino ethnic groups in the United States (31).

Among those subjects with asthma with the GG/GC genotypes at SNP +1437, there was a difference of 8.6 percentage points of predicted baseline FEV₁ between those subjects who were exposed to ETS and those who were not exposed to ETS. Because the FEV₁ was measured at least 8 hours after the use of inhaled β -agonists, this value is presumably an acceptable index of asthma severity. NHLBI guidelines currently use FEV₁ as an objective measurement in grading asthma severity. FEV₁ has been validated as a measure of airways obstruction because it closely correlates with pathologic scores of airway diameter (32) and decreased measures of FEV₁ were shown to be associated with the risk of future attacks and response to therapy among children with asthma (33, 34). Despite its usefulness, there is growing evidence to suggest that measures of FEV₁ may underestimate asthma severity in children and therefore may not be accurate for categorizing young patients with asthma (< 13 years) into mild, moderate, or severe persistent asthma categories (35). Symptom scores alone are unlikely to be a better measure of asthma severity because they are subjective and influenced by several factors. Recognizing that there is no single measure that accurately captures all facets of asthma severity, FEV₁ percent predicted has several advantages as a marker of asthma severity, including its objectivity and reproducibility (33, 36, 37).

The level of association between SNP +1437 and asthma severity was different among Mexicans and Puerto Ricans with asthma. There are several potential explanations for the observed differences. Concerning genetic association studies, self-identified ethnicity is a proxy variable for evolutionary history, patterns of linkage disequilibrium, and allele frequency differences. Although there may be genetic similarities between ethnic groups, they may differ with respect to environmental exposures, migration patterns, cultural factors, and a variety of other unmeasured factors, all of which can influence genetic associations. Specifically, the strength of association for different genes may vary across ethnic groups because the allele frequencies, linkage disequilibrium, and epistatic and environmental interactions are different in each group.

We found that associations between the SNP -159 genotypes and IgE levels are also dependent on environmental factors, such as ETS. Our results are consistent with results from some studies (13, 14, 17, 38, 39), but not others. (15, 16, 18). Baldini and colleagues (13) demonstrated the first association between CD14 and asthma-related phenotypes. However, Baldini and

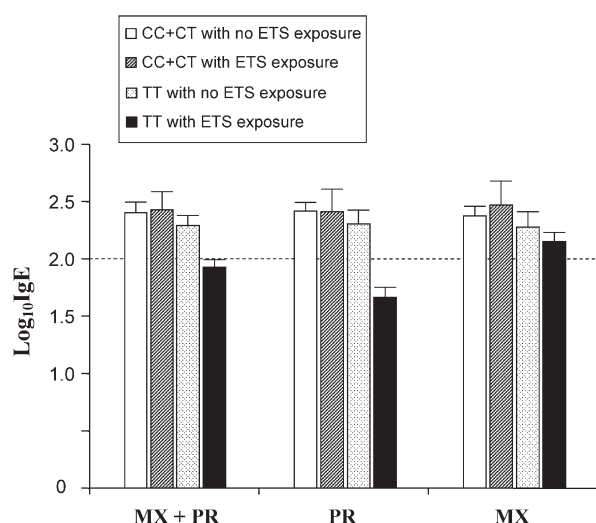


Figure 2. The plasma log₁₀ IgE levels by SNP -159 genotypes and ETS exposure. Probands with asthma with the TT genotype and exposed to ETS have significantly lower IgE levels compared with probands with the TT genotype and no ETS exposure or with the CC + CT genotype. Normal plasma IgE levels are those below the traced line.

colleagues found a strong association between CD14 variants and IgE levels in white subjects with asthma, but not in Hispanics with asthma. This ethnic-specific association may reflect differences in environment and/or genetic background or simply could be from study design and/or power. In their study, they analyzed 314 whites but only 89 Hispanics and 99 individuals of mixed ancestry, 90% of whom were white and Hispanic. Ober and colleagues (39) also demonstrated a strong association between CD14 and atopy using a family-based analysis among the Hutterites. Last, our results support and extend the results of Colilla and colleagues (6). Colilla and colleagues demonstrated that linkage to asthma on 5q31 was dependent on ETS exposure. Our results validate this observation by demonstrating that the association between the CD14 gene located in the 5q31, asthma severity, and IgE levels was also dependent on ETS exposure. Although our results are consistent with many studies, the results also differ from several reports in which no association was found between CD14 and asthma-related phenotypes. There may be several potential reasons for this discrepancy. First, we studied different populations. Racial and ethnic-specific genetic modifiers may modify genotypic relative risk (19). Linkage disequilibrium patterns are known to differ between populations and may influence genetic associations. Second, population stratification may also contribute to spurious associations (21, 40), a bias we avoided by performing family-based analysis. Third, another gene nearby in the 5q31 region, and not the CD14 gene, could be responsible for the gene-by-environment interaction. Last, different environmental factors may modify associations between the CD14 gene and asthma-related phenotypes (9). The present study would have failed to identify the significant association of the CD14 SNP -159 genotypes and IgE levels if we had not included exposure to ETS in our analysis.

CD14 is a pattern-recognition receptor and mediates efficient responses to endotoxins (LPS) (9). It has been proposed that exposure to endotoxins may modulate IgE regulation by activating innate immunity pathways that promote Th1 differentiation and/or suppress Th2-dependent IgE responses (7). IgE-mediated immune responses are an important factor in the immunopathology of asthma. There is a strong relationship between IgE levels and airway hyperresponsiveness, the physiologic hallmark of asthma (41). Bacterial endotoxin has been identified as an active component of cigarette smoke (10, 11). However, the mechanisms by which cigarette smoke may interact with the CD14 gene to influence IgE levels are not fully understood. Active smoking and exposure to secondhand smoke (ETS) has been shown to increase both the prevalence and severity of asthma (42, 43). Among children, *in utero* exposure, but not current exposure to tobacco, has been associated with a broad spectrum of wheezing outcomes among genetically susceptible children (44). Some studies suggest that ETS exposure results in increased IgE levels (45), whereas others suggest that ETS exposure or nicotine suppresses the immune system and may have a therapeutic benefit as an antiinflammatory agent (46). A study done in a rat model by Tulic and colleagues (47) demonstrated that early exposure to bacterial endotoxin suppresses allergic sensitization, but endotoxin administration to already sensitized animals exacerbates allergic inflammation. Therefore, it is possible that the interaction between ETS exposure and CD14 genetic variants may have different consequences depending on the stage of the disease at the time of the exposure. Our findings suggest that, among subjects with asthma who have the TT genotype at SNP -159, ETS exposure during the first 2 years of life results in decreased IgE levels.

Our study has several important limitations. First, we did not measure cotinine levels but rather ascertained active and passive smoking history by self-report, which is subject to recall bias.

However, we took measures to minimize this possibility. A trained interviewer, who was bilingual in Spanish and English, interviewed each family. Despite this, there is evidence that misclassification of smokers as nonsmokers may occur at a higher rate among nonwhite persons (48). These factors increase the potential for misclassification of exposure to ETS. However, recall bias would be independent of the CD14 genotype and would likely bias the results toward the null hypothesis. In addition, it is possible that Mexicans and Puerto Ricans with asthma are exposed to different types of tobacco smoke as are whites and blacks (49, 50). Specifically, there may have been differences in the type of tobacco exposure between subjects recruited in the continental United States versus those recruited in Puerto Rico or Mexico. Even among those subjects recruited in the United States, tobacco exposure may have varied and included mentholated, nonmentholated, or even hand-rolled cigarettes. In addition, the cross-sectional design of our study limited our ability to determine precisely the duration of ETS exposure and specifically the effects of *in utero* versus postnatal exposure; thus, longitudinal studies are warranted. Finally, we have performed a large number of comparisons, and replication of our results would be of value.

Another important limitation is the fact that there are no published pulmonary function reference values for Puerto Ricans. This limitation highlights the need for more research in minority populations. We used the predictive equations of Hankinson, the most extensive dataset of normal spirometric values for Latinos, including children. However, these reference values do not distinguish among the different Latino ethnicities. Although this is an important limitation, it does not alter our main finding of lower FEV₁, which was observed in both populations. When we analyzed the data using raw values instead of percent-predicted values, the results were the same. This consistency reinforces the validity of our results.

Finally, without a healthy control group, we cannot definitively say whether the association between CD14 genetic variants and FEV₁ level among subjects with asthma exposed to ETS is specific to individuals with asthma or to all persons regardless of asthma status. LeVan and colleagues (51) demonstrated that, among nonsmoking, adult male farmers (past or present), those who were genotype CD14 -159 TT (*n* = 19) had significantly lower lung function, as measured by FEV₁ and forced expiratory flow, midexpiratory phase, than farmers with the C allele (51). Also, farmers with the CD14 -1619 GG genotype (*n* = 11) were associated with lower lung function compared with farmers with the A allele. However, they could not differentiate whether the effect was determined by the -159T allele or the -1619 G allele. The authors have previously demonstrated that the -159 TT genotype is associated with higher levels of soluble CD14 (13). They hypothesized that elevated levels of soluble CD14 may result in increased responsiveness to environmental endotoxin and therefore increased levels of inflammation and airway obstruction.

We have compared these data to our results. However, there are several notable differences in our respective study designs, populations, and phenotypes, and therefore it is difficult to compare and contrast results. For example, LeVan and colleagues studied 97 healthy subjects, 50% of whom were of German descent, and they analyzed promoter variants, skin-prick tests, and pulmonary function. In contrast, we analyzed qualitative and quantitative measures of asthma, asthma severity, bronchodilator responsiveness, and IgE levels in 659 Puerto Rican and Mexican probands with asthma and their parents (*n* = 1,977). In addition to promoter variants, we analyzed an SNP in the 3' UTR.

Global consumption of cigarettes is rising steadily (52). Although cigarette consumption is decreasing in some countries,

more people are smoking worldwide and average cigarette consumption per smoker is also increasing (52). Identification of gene-by-environment interactions in asthma may lead to new insights into asthma pathogenesis and treatment. Children are at particular risk from adults' smoking. The frequencies of the genetic susceptibility CD14 -159 C and +1437 G alleles are as high as 55% of the general population. The large size of the "at-risk population" underscores the significance of this observation for the health and well-being of everyone.

Conflict of Interest Statement: S.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; P.C.A. has received \$250,000 from GlaxoSmithKline in 2004–2006 as a research grant to study asthma exacerbations; S.N. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; N.U. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.R.R.-S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; H.-J.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; A.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.T. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.A. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; I.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.K.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.L. has received lecture fees during 2003–2004 from Merck; H.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; R.A.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; M.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; S.T.W. received a grant for \$900,065, Asthma Policy Modeling Study, from AstraZeneca from 1997–2003. He has been coinvestigator on a grant from Boehringer Ingelheim to investigate a chronic obstructive pulmonary disease (COPD) natural history model, which began in 2003. He has received no funds for his involvement in this project. He had been an advisor to the TENOR Study for Genentech and has received \$5000 for 2003–2004. He received a grant from Glaxo-Wellcome for \$500,000 for genomic equipment for 2000–2003. He was a consultant for Roche Pharmaceuticals in 2000 and received no financial remuneration for this consultancy; J.G.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; J.M.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; W.R.-C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; R.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; E.K.S. received grant support and honoraria from GlaxoSmithKline for a study of COPD genetics. He also received a \$500 speaker fee from Wyeth for a talk on COPD genetics; E.G.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank the families and the patients for their participation. The authors also thank the numerous health care providers and community clinics for their support and participation in the GALA study. In addition to the primary clinical centers of the investigators, participating community clinics and hospitals include the following: La Clinica de La Raza, Oakland, CA; UCSF—Children's Hospital of Oakland Pediatric Clinical Research Center, Oakland, CA; General Clinical Research Center, SFGH, San Francisco, CA; Alliance Medical Center, Healdsburg, CA; Santa Clara Valley Medical Center, San José, CA; Fair Oaks Family Health Center, Redwood City, CA; Clinica de Salud del Valle de Salinas, Salinas, CA; Natividad Medical Center, Salinas, CA; Asthma Education and Management Program, Community Medical Centers, Fresno, CA; Diagnostic Health Centers of Corozal, Naranjito, Catano, Orocovis, Barranquitas, and San Antonio Hospital, Mayagüez, PR; Morris Heights Health Center, Bronx, NY; Paterson School Board, Paterson, NJ; Eva's Clinic, Paterson, NJ; Lincoln Medical Center, Bronx, Harlem Hospital Center, NY; Harlem Hospital Center, NY; and the Metropolitan Hospital Center, New York, NY. The authors also thank Carmen Jimenez, Yannett Marcano, Pedro Yapor, M.D., Alma Ortiz, M.D., Lisandra Perez, M.D., and Sheila Gonzalez, M.D., for their assistance with recruitment. Finally, the authors especially thank Dean Sheppard for all of his support in the creation and execution of the GALA study.

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