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ORIGINAL RESEARCH

Polymorphism in Osteopontin Gene (*SPP1*) Is Associated with Asthma and Related Phenotypes in a Puerto Rican Population

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Recent studies have shown that osteopontin, a cytokine with suggested immunoregulatory functions, may contribute to pathogenesis of asthma. To determine whether single-nucleotide polymorphisms (SNPs) in *SPP1*, the gene encoding osteopontin, are associated with risk of asthma, we genotyped 6 known SNPs in *SPP1* in the well-characterized Genetics of Asthma in Latino Americans population of 294 Mexican and 365 Puerto Rican parent–child asthma trios. The associations between SNPs and asthma or asthma-related phenotypes were examined by transmission disequilibrium tests as implemented in the family-based association test program. Three polymorphisms, 1 in exon 7 (rs1126616C) and 2 in the 3′-untranslated region (rs1126772A and rs9138A) of *SPP1*, were associated with diagnosis of asthma, severity of asthma, asthma in subjects with elevated immunoglobulin E (IgE) (IgE >100 IU/mL), and postbronchodilator FEV_1 in Puerto Ricans (P values $=0.00007$–0.04). The CC genotype of rs1126616 conferred an odds ratio of 1.7 (95% CI = [1.3, 2.3], P value adjusted for multiple comparisons $=0.001$) for asthma compared with the CT and TT genotypes. Furthermore, haplotype analysis identified rs1126616C-rs1126772A-rs9138A to be associated with an increased risk for asthma, severity of asthma, and asthma in subjects with elevated IgE ($P=0.03$). There was no association between the *SPP1* SNPs and asthma outcomes in Mexicans. Our findings suggest that the *SPP1* gene is a risk factor for asthma and asthma-related phenotypes in Puerto Ricans, and are consistent with previous animal and human studies on the role of osteopontin in pathogenesis of asthma.

Introduction

Osteopontin is a small integrin-binding ligand N-linked glycoprotein and a cytokine with suggested diverse roles in tissue remodeling, fibrosis, immunomodulation, inflammation, and tumor metastasis.[1–6] Although it is synthesized at the highest levels in bone, it is also made by a variety of other cells including epithelial cells, smooth muscle cells, and immune cells such as macrophages and T cells that populate the airways.[7–14]

Recent evidence suggests that osteopontin may play a role in the pathogenesis of asthma. Several investigators have shown that osteopontin plays an important role in the pathophysiology of murine models of allergic airway disease. Osteopontin deficiency, either through administration of blocking antibody or genetic deficiency (knockout mice),

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has been reported to be protective against airway hyperresponsiveness (AHR) and airway remodeling in these murine models.[15–19] In humans, immunohistochemistry of endobronchial biopsies has shown increased osteopontin expression in bronchial epithelial cells and subepithelial inflammatory cells in asthmatic subjects compared with nonasthmatic controls.[15] In addition, several recent studies have reported an increased level of osteopontin in bronchoalveolar lavage fluid and induced sputum in asthmatic subjects.[20–22] A recent genetic association study in a Japanese population has reported an association between genetic variant in the osteopontin gene, *SPP1*, and total serum immunoglobulin E (IgE) levels in nonasthmatic subjects, thus suggesting that osteopontin may participate in the regulation of basal IgE production.[23]

Based on these observations, we hypothesized that putative functional polymorphisms in *SPP1* is a risk factor for asthma and asthma phenotypes. To test this hypothesis, we examined the relationship between 6 known polymorphisms in *SPP1* and asthma, asthma severity, lung function, and IgE level in 2 Latino populations from the well-characterized family-based population of the Genetics of Asthma in Latino Americans (GALA) study.[24]

Materials and Methods

Study population

The subjects included in the genetic study are part of the GALA study and have been previously described.[24] Subjects with asthma and their biological parents were enrolled over a 4-year period in the San Francisco Bay Area, California; New York City, New York; Puerto Rico; and Mexico City, Mexico. Asthmatic subjects were enrolled only if all 4 biological grandparents were of the same ethnic backgrounds: Puerto Rican in New York and Puerto Rico, and Mexican in San Francisco and Mexico City. Ethnicity was self-reported. Subjects were included if they were 8–40 years of age, had a current physician diagnosis of asthma or an improvement in FEV_1 of $>12\%$ after administration of albuterol, and reported asthma symptoms (wheezing, cough, or shortness of breath) over the 2 years before enrollment.

Subjects were excluded if they had a 10 pack-year or greater smoking history, if they had a medical contraindication to participation, or if pregnant, were in the third trimester. Recruitment criteria were identical at each site. The study protocol was approved by institutional review boards at each of the participating sites. All subjects provided written, informed consent. Minors provided age-appropriate assent.

Pulmonary function test, severity of asthma, and IgE measurement in the GALA population

Pulmonary function tests were expressed as a percentage of the predicted normal value by using age-adjusted Mexican-American prediction equations from Hankinson.[25] Lung function was measured before and after administration of bronchodilator [albuterol, 180 μg (2 puffs) for subjects <16 years old, or 360 μg (4 puffs) for subjects ≥ 16 years old]. We used a measurement of the percent predicted FEV_1 after bronchodilator administration (pos-bronchodilator FEV_1) to test for association between *SPP1* polymorphisms and lung function. Details of GALA pulmonary function testing are described elsewhere.[24]

The severity of asthma was classified as mild or moderate-severe based on a modified version of the American Thoracic Society–Division of Lung Diseases Epidemiology Questionnaire as previously described.[24] Subjects were classified as having moderate-to-severe asthma if they met any of the following criteria: (1) They had daily asthma symptoms for 3 or more months of the previous year, regardless of medications usage; (2) they had nocturnal asthma symptoms >1 night per week for 3 or more months of the previous year; (3); they had a prebronchodilator $FEV_1 < 80\%$ of race-corrected predicted normal value (measured at the study visit).

Total plasma IgE was measured in duplicate by using Uni-Cap technology (Pharmacia). Subjects were classified as having elevated IgE level if their total IgE level was greater than or equal to 100 IU/mL per current interpretation of total serum IgE values in screening for atopic diseases in adults.[26,27]

Selection and genotyping of SPP1 polymorphisms

The *SPP1* gene is located on chromosomal region 4q21-q25 and is ~7.8kb in length with 7 exons. Exon 1 and a small portion of exon 2 code for the 5′-untranslated region (UTR), whereas a portion of exon 7 codes for the 3′-UTR of the *SPP1* gene transcript. Selection of polymorphisms in the *SPP1* gene for genotyping in the GALA population was done by using the HapMap phase I, II, and III data (http://hapmap.org/cgi-perl/gbrowse/hapmap24_B36/). All reported polymorphisms were selected for genotyping if they met the following criteria: (1) They were in the exonic region of the *SPP1* gene, and (2) they had a minor allele frequency of $>5\%$ in all HapMap populations (Caucasian, Yoruban, and combined Chinese and Japanese). Six polymorphisms within the *SPP1* gene met these criteria: rs6812524, rs7435825, rs1126616, rs4660, rs4754, rs1126772, and rs9138. Since polymorphisms rs4754 and rs9138 were in tight linkage disequilibrium (LD) ($r^2 > 0.8$) in all HapMap populations, we selected rs9138 for genotyping. A sample size of 300 parent–child asthma trios provides $>80\%$ power to detect underlying disease locus with allele frequency as low as 5% and odds ratio (OR) of 1.5 or more.

Genotyping of the selected polymorphisms in the GALA population was performed by using multiplex PCR assays followed by single-base primer extensions using iPLEX enzyme and buffers (Sequenom). Primer extension products were measured with the MassARRAY Compact System (Sequenom), and mass spectra were analyzed by using TYPER software (Sequenom) to generate genotype calls. Quality control was performed on the genotype calls for GALA Puerto Rican and Mexican asthma trios. Genotype call rates were generally high ($>90\%$) and reproducible.

Genetic analysis

Mendelian inconsistencies were identified by using PEDCHECK. Families with Mendelian inconsistencies were excluded from further analysis. Hardy–Weinberg equilibrium (HWE) for genotypes and allele frequency at each locus were calculated by means of χ^2 goodness-of-fit tests, separately for Puerto Ricans and Mexicans. The HWE was calculated separately for parents and probands within each

ethnic group. Family-based association test (FBAT) and HaploFBAT were used to assess the association between individual polymorphisms and haplotypes, respectively, with asthma and quantitative measures of asthma-related phenotypes in the GALA trios. A dominant genetic model was used for the analyses to provide greater statistical power by generating tests with a few degrees of freedom and allowing for genetic tests for polymorphisms with low minor allele frequency (Supplementary Table S1; Supplementary Data is available online at www.liebertonline.com/ped). The quantitative phenotypes analyzed include baseline lung function (measured as prebronchodilator FEV_1, % of predicted), bronchodilator responsiveness (measured as ΔFEV_1, relative percent of predicted), and log transformed IgE level. All analyses were performed by using the statistical software package STATA/SE 9.0 (STATA Corporation).

Correction for multiple testing

The correction for multiple testing was performed using SNPSpD (an effective Bonferroni-type correction). Each phenotype (asthma, Postbronchodilator FEV_1, and $Log_{10}IgE$) in the present study was tested for 6 single-nucleotide polymorphisms (SNPs). However, unlike in the HapMap populations, the SNPs were in partial LD in the GALA population (see Results section), SNPSpD found the 6 SNPs to be equivalent to 4 independent SNPs. Thus, the level of significance α for *SPP1* association tests was set at 0.004 [0.05/(4*3)], where 4 is the number of effective independent SNPs and 3 is the number of phenotypes tested. Since Mexicans and Puerto Ricans are 2 separate populations and could have different risk alleles; we did not correct the analysis within populations for 2 tests. Since subgroup analysis was undertaken only in cases where there was an overall association, they were not corrected for multiple testing.

Results

Characteristics of GALA subjects for SPP1 genetic analysis

Under GALA, 365 Puerto Rican and 294 Mexican parent–child asthma trios (an asthmatic child and nonasthmatic parents) were recruited. Demographic and clinical characteristics of subjects are shown in Table 1. The median ages of the Puerto Rican and Mexican asthmatics were 12.0 and 13.3 years, respectively. The median baseline lung function (prebronchodilator FEV_1) was 83% and 89% for Puerto Rican and Mexican asthmatics, respectively, and the bronchodilator responsiveness measured by the change in FEV_1 (ΔFEV_1) was significantly greater for Mexican subjects with asthma than for Puerto Rican subjects with asthma ($P < 0.0001$) as previously described.[24]

Allele frequencies, HWE, and LD

The minor allele frequencies of *SPP1* polymorphisms in Puerto Rican and Mexican probands and parents are listed in Table 2. The observed distribution of genotypes within each ethnic group was in HWE in the parents. All 6 polymorphisms had minor allele frequencies of >2% in Puerto Ricans, whereas in Mexicans, only 4 out of 6 had a minor allele frequency >2%. No significant pairwise LD was observed between *SPP1* polymorphisms ($r^2 \leq 0.8$) except for rs1126616 and rs9138 in both Puerto Ricans and Mexicans, although in the HapMap populations these 2 polymorphisms were not in tight LD ($r^2 \leq 0.8$) (Table 3).

Table 1. Characteristics and Pulmonary Function Results of Genetics of Asthma in Latino Americans Asthmatic Probands (Puerto Ricans and Mexicans) Included in the *SPP1* Genetic Analysis

	Ethnicity		
	Puerto Ricans (n = *365*)	*Mexicans* (*n* = *294*)	P *value*
Characteristic			
Age (years)	12.0 [10, 15]	13.3 [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
Serum IgE (IU/mL)	258.5 [92, 628]	270.0 [99–615]	0.92
Moderate–severe asthma (%)	67.1%	66.7%	0.90
ETS exposure (%)	41.0%	40.8%	0.58
Baseline spirometry			
Pre-BD FEV_1 (% predicted)	83 [74, 93]	89 [77, 100]	<0.0001
Post-BD FEV_1 (% predicted)	89 [80, 99]	97 [86,108]	<0.0001
Bronchodilator responsiveness			
ΔFEV_1 (relative% predicted)	5.0 [0.6, 10]	7.4 [4, 12]	<0.0001

Values are expressed as median [interquartile range]. Analysis was done by Mann–Whitney rank test.

n, number of probands and families; BMI, body mass index; Pre-BD, baseline lung function before administration of bronchodilator; Post-BD, post-bronchodilator lung function; ΔFEV_1, the relative percent change in Pre-BD FEV_1 after albuterol administration.

IgE, immunoglobulin E.

Association analysis of SPP1 polymorphisms with asthma and related phenotypes

Polymorphisms with minor allele frequency <5% were analyzed for genetic associations (6 SNPs for Puerto Ricans and 3 SNPs for Mexicans). Among Puerto Ricans, rs1126616, a synonymous polymorphism in exon 7, and rs1126772 and rs9138, 2 SNPs in the 3′-UTR were significantly associated with asthma ($P = 0.00007$–0.04) (Table 4). The CC genotype of rs1126616 conferred an OR of 1.7 (95% CI = 1.3–2.3, $P = 0.00007$) for asthma compared with the CT and TT genotypes. Subgroup analysis revealed a consistent association in both individuals with normal and elevated levels of IgE as well as in both mild and moderate/severe asthma ($P = 0.00008$–0.04, Table 4). After correction for multiple testing, the association for rs1126616 with presence of asthma in Puerto Ricans remained statistically significant ($P_{adjusted} = 0.001$). The CC genotype of rs1126616 was significantly associated with lower lung function (postbronchodilator FEV_1) in Puerto Ricans ($P = 0.02$). However, this association did not remain significant after correction for multiple testing. Since rs1126772 and rs9138 are in partial and tight LD with rs1126616, respectively, similar association results with asthma, asthma severity, asthma in individuals with elevated IgE, and lung function were obtained for these 2 polymorphisms (Table 4). No significant association between

Table 2. *SPP1* Polymorphism Reference (rs) Number, Location, Alleles, Minor Allele, and Its Frequency in Genetics of Asthma in Latino Americans Puerto Rican and Mexican Probands and Parents

rs number	Chromosomal position	Location within the Gene	Alleles (amino acid change)	Minor allele	Minor allele frequency (%)			
					Puerto Rican		Mexican	
					Probands	Parents	Probands	Parents
rs6812524	89121749	Exon 6	G/A (Ser-Ser)	A	5.8%	5.9%	0.08%	0.08%
rs7435825	89122798	Exon 7	G/A (Ser-Asn)	A	4.4%	4.1%	0.05%	0.05%
rs1126616	89122877	Exon 7	C/T (Ala-Ala)	T	32.3%	31.7%	44.3%	40.3%
rs4660	89123029	Exon 7	G/A (Arg-His)	A	2.4%	2.7%	0.03%	0.03%
rs1126772	89123210	3′-UTR	A/G	G	21.2%	22.0%	22.8%	22.6%
rs9138	89123366	3′-UTR	A/C	C	31.1%	33.2%	42.2%	41.9%

UTR, untranslated region.

SPP1 genotypes and log IgE level was found in Puerto Ricans. In contrast to Puerto Ricans, there was no significant association between *SPP1* polymorphisms and asthma or related phenotypes in Mexicans. There was statistically significant heterogeneity for the association between rs1126772 and asthma in the 2 ethnic groups ($P_{het} < 0.03$).

Association analysis of SPP1 haplotypes with asthma and related phenotypes

A total of 6 different *SPP1* haplotypes (frequency of >1%) were observed in Puerto Ricans. The haplotype association results were consistent with those of the individual polymorphisms (Table 5). Among Puerto Ricans, the common haplotype that carried C, A, and A alleles for rs1126616, rs1126772, and rs9138, respectively, was associated with increased risk for asthma ($P = 0.04$), but showed only a trend toward association with severity of asthma ($P = 0.09$) and asthma in subjects with elevated IgE ($P = 0.05$). We also observed a trend toward association between the common haplotype carrying T, G, and C alleles for rs1126616, rs1126772, and rs9138, respectively, with lower log IgE level ($P = 0.09$).

Discussion

In this candidate gene study of 2 independent Latino populations, we found that polymorphisms in osteopontin gene, *SPP1*, are associated with the presence of asthma, severity of asthma, and asthma in subjects with elevated IgE in Puerto Ricans. In addition, haplotype analysis revealed that the 3 SNPs in the exon 7 and 3′-UTR explain the observed association with asthma and asthma phenotypes in Puerto Ricans, thus supporting the hypothesis that the causal variant of *SPP1* might be near its 3′-UTR, the region that could affect the regulation of *SPP1* expression. We did not find any association between the *SPP1* SNPs and asthma outcomes in Mexicans.

Previous animal studies have shown that osteopontin contributes to the development of airway remodeling and AHR in murine models of experimental asthma.[16–18] Further, several human studies have suggested that osteopontin level is increased in airway of asthmatic subjects and is associated with various asthma phenotypes.[15,20–22] Additionally, the chromosomal region of 4q24 where *SPP1* gene is located (4q21-q25) has been reported to be associated with atopy and with atopic disease in asthmatic subjects.[28,29] Together, these studies suggest that the osteopontin gene, *SPP1*, is a reasonable candidate gene for asthma susceptibility. Our finding of significant association between *SPP1* polymorphisms and asthma, asthma severity, and atopic asthma in Puerto Ricans is consistent with a possible role of osteopontin in pathogenesis of asthma.

The only published study that has investigated genetic association of *SPP1* with total serum IgE levels, atopy, and asthma was performed in a Japanese population. The Japanese investigators found a significant association between *SPP1* polymorphisms and total IgE in nonasthmatic subjects but not among subjects with asthma. In addition, they did not find an association between *SPP1* polymorphisms and asthma or atopy. In our study, we found a trend toward an association ($P = 0.09$) between IgE level and *SPP1* haplotype in our Puerto Rican asthmatics (see Table 5). The haplotype that was associated with lower IgE level had alleles for polymorphisms rs1122616, rs1126772, and rs9138 that were protective for asthma. It is possible that,

Table 3. Pairwise Linkage Disequilibrium Measure r^2 Between *SPP1* Polymorphisms in Puerto Ricans (Top Triangle) and Mexicans (Bottom Triangle)

	r^2					
Puerto Rican	rs6812524	rs7435825	rs1126616	rs4660	rs1126772	rs9138
rs6812524		0.70	0.03	0.00	0.02	0.02
rs7435825	0.60		0.02	0.00	0.01	0.02
rs1126616	0.01	0.00		0.01	0.58	**0.92**
rs4660	0.00	0.00	0.00		0.01	0.01
rs1126772	0.00	0.00	0.41	0.00		0.55
rs9138	0.01	0.00	**0.97**	0.00	0.39	
Mexican	rs6812524	rs7435825	rs1126616	rs4660	rs1126772	rs9138

Significant LD ($r^2 > 0.8$) are in bold.

Table 4. Family-Based Association Analysis of Asthma and Related Traits with *SPP1* Polymorphisms in Puerto Rican Asthma Trios

	SPP1 *polymorphism (genotype)*					
	rs6812524 (GG)	*rs7435825 (GG)*	*rs1126616 (CC)*	*rs4660 (GG)*	*rs1126772 (AA)*	*rs9138 (AA)*
	P *values (direction of association*[a]*)*					
Asthma	0.50 (+)	0.51(+)	**0.00007 (+) [0.0008]**	0.46 (+)	**0.04 (+)** [0.5]	**0.03 (+)** [0.4]
Moderate-severe asthma	0.54 (+)	0.54 (+)	**0.0004 (+) [0.005]**	0.48 (+)	0.34 (+)	0.10 (+)
Asthma in subjects with elevated IgE (>100 IU/mL)	0.82 (+)	0.73 (+)	**0.00008 (+) [0.001]**	0.74 (−)	**0.02 (+)** [0.2]	**0.01 (+)** [0.1]
Post-BD FEV_1	0.29 (+)	0.32 (+)	**0.02 (−)** [0.2]	0.46 (+)	0.07 (−)	0.07 (−)
Log_{10} IgE Level	0.41(+)	0.72 (+)	0.18 (+)	0.27 (−)	0.14 (+)	0.16 (+)

[a]"+" and "−" indicate direction of association. "+" indicates increased risk for asthma or higher value of the quantitative phenotypes Post-BD FEV_1 and log IgE, whereas "−" indicates a decreased risk for asthma and lower value of the quantitative phenotypes. *P* values <0.05 are in bold. When the *P* values are nominally significant, the *P* values, adjusted for multiple comparisons, are also given in brackets.

similar to the Japanese study, the association between *SPP1* polymorphisms and IgE level is even stronger in nonasthmatic Puerto Ricans. However, our parent–child trio study design, where phenotype information is not collected on nonasthmatic children, precludes such an analysis. A logical extension to this study would be to test the association between *SPP1* haplotype and IgE levels in a nonasthmatic Puerto Rican population.

Our findings are also consistent with the suggested immunoregulatory role of osteopontin.[30] Previous studies have shown *SPP1* polymorphisms to be significantly associated with the development and/or disease activity of several immune-mediated inflammatory diseases, including multiple sclerosis, systemic lupus erythematosus (SLE), hepatitis C, and autoimmune lymphoproliferative syndrome (ALPS). *SPP1* polymorphisms in the coding (rs1122616) and 3′-UTR (rs1126772 and rs9138) regions that were associated with asthma and asthma-related phenotypes in our study have previously been implicated in autoimmune diseases including SLE and ALPS. Interestingly, the alleles that we found to be risk factors for the development of asthma (a Th2 disease) and asthma-related phenotypes in our study were protective against SLE and ALPS (Th1 diseases). This is consistent with the suggested biological role of osteopontin in the regulation of Th1/Th2 balance.[1,30–32]

Osteopontin interacts with multiple cell surface integrins including αvβ3 integrin through its Arg-Gly-Asp (RGD) motif, and binds directly to the β3 subunit of integrins.[33] To explore potential pathways through which polymorphisms in osteopontin may contribute to pathogenesis of asthma, we searched the HuGE Gene Navigator[34] for studies of *ITGB3*, the gene encoding the β3 integrin subunit and asthma. Weiss et al. first found that variation in ITGB3 was associated with asthma and sensitization to mold allergen in 4 populations.[35] The association was then confirmed in 2 other studies, including one based on genomewide association data.[36,37] This raises the intriguing possibility of a gene-gene interaction between *SPP1* and *ITGB3*. A pathway analysis of osteopontin shows that it inhibits the matrix metalloproteases (MMP) 2 and 9. Although *MMP2* has not been associated with asthma, several studies have found an association between *MMP9* and asthma.[38,39] Osteopontin has also been associated with the nuclear factor kappa-B (NFkB) and tumor necrosis factor (TNF-α) pathway; the latter is well known to be associated with asthma,[40,41] with 66 reported genetic association studies in the HuGE navigator.[34]

Table 5. Family-Based Association Analysis of Asthma and Related Traits with SPP1 Haplotypes in Puerto Rican Asthma Trios

	SPP1 haplotypes[a] *(frequency)*					
	G.G.C.G.A.A (57.7%)	*G.G.T.G.G.C (21.5%)*	*G.G.T.G.A.C (10.5%)*	*A.A.C.G.A.A (4.0%)*	*G.G.C.A.A.A (2.6%)*	*A.G.C.G.A.A (1.6%)*
	P *values (direction of association*[b]*)*					
Asthma	**0.04 (+)**	NS	NS	NS	NS	NS
Moderate-Severe Asthma	0.09 (+)	NS	NS	NS	NS	NS
Atopic Asthma	0.05 (+)	NS	NS	NS	NS	NS
Post-BD FEV_1	NS	NS	NS	NS	NS	NS
Log_{10} IgE Level	NS	0.09 (−)	NS	NS	NS	NS

[a]Order of the polymorphisms in the haplotypes is rs6812524, rs7435825, rs1126616, rs4660, rs1126772, and rs9138. Only haplotypes with frequency >2% were included in the association analysis.

[b]"+" and "−" indicate direction of association. "+" indicates increased risk for asthma or higher value of the quantitative phenotypes Post-BD FEV_1 and log IgE, whereas "−" indicates decrease risk for asthma and lower value of the quantitative phenotypes.

NS, not significant with *P* value >0.1.

P-values <0.05 are in bold.

The most significantly associated polymorphism of the *SPP1* gene in our study is a synonymous polymorphism and does not cause any change in the osteopontin protein amino acid sequence. Although the neutral theory of molecular evolution holds that synonymous mutations in exons have no significant effect on selection, studies in lower organisms with large effective population sizes have shown that in highly expressed genes, protein synthesis is optimized by biasing the usage of synonymous codons to match the abundance of tRNA, a phenomenon known as codon usage bias.[42] Although this theory is controversial in mammalians,[43] synonymous polymorphisms can still lead to phenotypic changes (such as asthma and asthma-related phenotypes) through their effect on mRNA stability or processing,[44] as previously documented for both synonymous and nonsynonymous polymorphisms in genes such as *DRD1*, *ABCB1*, and *OPRM1*, or by disrupting the splicing process.[45] Alternatively, the polymorphism could be in tight LD with one or more other SNPs that may have important structural and functional consequences. Further sequencing of the *SPP1* gene in this population of Puerto Ricans would help elucidate this possibility.

It is interesting that although we found a robust association between *SPP1* polymorphisms and asthma in a Puerto Rican population, the same finding was not seen in Mexicans. Although it is always possible that such findings are spurious, the study was adequately powered to observe an effect in Mexicans of the magnitude seen in Puerto Ricans. Differences in asthma genetic associations between racial/ethnic groups have been found in a recent asthma meta-analysis,[46] and we have documented such differential effects between the 2 populations in candidate gene studies,[47] and may be due to a number of reasons. First, although both Latino populations are admixed descendents of European, African, and Native American populations, the relative proportions of those ancestral populations differ between the contemporary Puerto Rican and Mexican populations. We have previously demonstrated that genetic ancestry can modify genetic associations.[48] Therefore, the observed differences in genetic association may be due to effect modification by genetic ancestry interactions. Alternatively, there may be differential gene-environment interactions. Osteopontin expression and secretion in alveolar macrophages is significantly increased in smokers as compared with nonsmoker control subjects.[49] This suggests the possibility of an interaction between exposure to tobacco smoke and *SPP1* polymorphisms. In addition, other unmeasured environmental factors, such as ambient outdoor air pollution or indoor wood smoke exposure, could modify the effect of *SPP1* polymorphisms and asthma. Systematic differences in an unmeasured environmental exposure between Mexican and Puerto Rican children could explain the differences in effect magnitude observed if *SPP1* polymorphisms made children susceptible to environmental insults that induce asthma.

Our group has previously reported that Puerto Rican children have a lower response to albuterol than do Mexican children.[24] This may suggest that a larger component of airway obstruction in Puerto Rican asthmatics is fixed, and, therefore, a lower percentage is due to reversible bronchoconstriction. These results are consistent with the suggested role of osteopontin in airway remodeling and reported observation that increased induced sputum osteopontin levels are associated with a lower postbronchodilator FEV_1,[50] and suggest a rationale to investigate a possible role for *SPP1* polymorphisms, airway osteopontin levels, and lung function in Latino populations. In this study, we found a nominal association between rs1126616 and lower postbronchodilator FEV_1 ($P = 0.02$), in the Puerto Rican population, though this result was not significant after adjustment for multiple comparisons. This suggests that Puerto Ricans with asthma who have the CC genotype have a greater degree of fixed airway obstruction, consistent with the role of osteopontin in airway remodeling. If it is borne out that *SPP1* polymorphisms are associated with differences in airway osteopontin levels and response to albuterol, then this would offer a plausible explanation for the differences in bronchodilator response between Mexicans and Puerto Ricans we had previously described.

There were several limitations to this study. Although the finding was robust in the Puerto Rican population, we have not replicated the findings in an independent population. Such replications by an independent group would be important to verify the association between *SPP1* and asthma. In addition, we were only able to evaluate 6 exonic SNPs. These SNPs were chosen, because they had been previously investigated in the literature and were more likely to be mechanistically relevant to the genetic association. Although noncoding SNPs have commonly been implicated in genetic associations with asthma, genotyping more SNPs would have reduced the study's power due to the need to adjust for a greater number of comparisons. However, it is important to note that our study would not preclude an association between asthma and SNPs apart from rs1126616, especially in other populations. Finally, although there is substantial evidence linking osteopontin with asthma, a functional study, establishing an association between *SPP1* polymorphisms and osteopontin levels, is beyond the scope of this paper. We hope that the publication of this genetic association study in humans will spur other groups to examine the mechanism by which polymorphisms in the *SPP1* gene may contribute to pathogenesis of asthma.

In conclusion, our genetic association study of a candidate gene, osteopontin, suggests that *SPP1* may be a genetic risk factor for development of asthma and asthma-related phenotypes in Puerto Ricans, and is consistent with data from several animal and human studies suggesting a role for osteopontin in pathogenesis of asthma. Further studies to replicate and validate these associations in Puerto Rican and other populations as well as structural and functional studies of osteopontin should improve our understanding of its role in asthma pathogenesis.

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Author Disclosure Statement

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