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PAI-1 gain-of-function genotype, factors increasing PAI-1 levels, and airway obstruction: the GALA II Cohort

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Michael G. Sherenian conceptualized the report, performed some analyses, drafted the initial manuscript and approved the final manuscript as submitted.

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Lewis J Smith and Ravi Kalhan participated in the conceptualization and design of this report, reviewed and revised the manuscript and approved the final manuscript as submitted.

Esteban G. Burchard (GALA II PI) designed the GALA II study, obtained funding for performance of the study, supervised performance of the study at all sites, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Abstract

Background—PAI-1 gain of function variants promote airway fibrosis, and are associated with asthma and with worse lung function in subjects with asthma.

Objective—We sought to determine if the association of a gain-of-function polymorphism in Plasminogen Activator Inhibitor −1 (PAI-1) with airway obstruction is modified by asthma status, and whether any genotype effect persists after accounting for common exposures that increase PAI-1 level.

Methods—We studied 2070 Latino children (8–21y) with genotypic and pulmonary function data from the GALA II cohort. We estimated the relationship of the PAI-1 risk allele with FEV1/FVC by multivariate linear regression, stratified by asthma status. We examined the association of the polymorphism with asthma and airway obstruction within asthmatics via multivariate logistic regression. We replicated associations in the SAPPHIRE cohort of African Americans (n=1056). Secondary analysis included the effect of the at-risk polymorphism on post bronchodilator lung function.

Results—There was an interaction between asthma status and the PAI-1 polymorphism on FEV_1/FVC (p=0.03). The gain-of-function variants, genotypes (AA/AG), were associated with lower FEV_1/FVC in subjects with asthma (β=−1.25, CI:−2.14,−0.35, p=0.006), but not in controls. Subjects with asthma and the AA/AG genotypes had a 5% decrease in FEV_1/FVC (p<0.001). In asthmatics, the risk genotype (AA/AG) was associated with a 39% increase in risk of clinically relevant airway obstruction (OR=1.39, CI:1.01, 1.92, p=0.04). These associations persisted after exclusion of factors that increase PAI-1 including tobacco exposure and obesity.

Conclusions and Clinical Relevance—The decrease in the FEV_1/FVC ratio associated with the risk genotype was modified by asthma status. The genotype increased the odds of airway obstruction by 75% within asthmatics only. Since exposures known to increase PAI-1 levels did not mitigate this association, PAI-1 may contribute to airway obstruction in the context of chronic asthmatic airway inflammation.

Introduction

Urban minority children are more likely than white children to have asthma and reduced lung function.[1,2] Genetic and environmental factors contribute to decreased lung function in this group. While lower socioeconomic status (SES) is correlated with decreased pulmonary function in children and with greater rates of decline in adulthood,[2–5] genetic ancestry is associated with lung function[6] in admixed populations even after accounting for SES.[7] These genetic associations may be population specific[8] or dependent on environmental factors to affect lung disease and phenotypes.[9,10] We recently studied a gain-of-function polymorphism in PAI-1, which is a variant whose frequency varies by African ancestry.[11] We found a joint effect of the PAI-1 polymorphism at the rs2227631 promoter region and early life infection, which was associated with reduced FEV_1 and FEV_1/FVC ratio in children with asthma.[10]

While there are a few studies of the association of PAI-1 and lung function in children with asthma,[12] there are no studies in generally healthy children without asthma and therefore without ongoing airway inflammation. This is a biologically relevant question since plasminogen activator inhibitor 1 (PAI-1) is important in deposition of extracellular matrix and airway remodeling after lung injury.[13,14] It is also unclear as to whether genotype associations with lung function would persist after accounting for environmental factors such as smoke exposure, obesity, and early viral illnesses, which are prevalent in urban minority populations,[15–18] and all of which increase PAI-1 levels.[19–22] Further, evaluating the role of factors which affect PAI-1 levels may be particularly important for urban minority Latino Americans who have approximately 65% higher PAI-1 protein levels compared to African Americans and non-Latino Caucasians.[23,24]

We sought to determine whether the association of a gain-of-function PAI-1 mutation with airway obstruction is modified by asthma status in Latino children in the Genes-environments & Admixture in Latino Americans (GALA II) cohort. We also sought to determine if the associations of the polymorphism persist after controlling for common chronic stimuli known to increase PAI-1 levels including second hand smoke exposure and obesity. We hypothesized that there would be an interaction between asthma status and the PAI-1 gain-of-function polymorphism on the outcome of airway obstruction, such that an association would be present in asthmatics only. We also hypothesized that this effect would be present even after controlling for common childhood exposures that are associated with elevated PAI-1 levels.

Materials and Methods

Subjects

The participants of this study were a population of Latino children enrolled as a part of the GALA II study. Since July 2006, a total of 4,045 subjects including 1,976 patients with asthma were recruited from urban study centers (Chicago, Illinois; Bronx, New York; Houston, Texas; San Francisco Bay Area, California; and San Juan, Puerto Rico) across the mainland U.S. and Puerto Rico through June 2011. Subjects were eligible if they were 8–21 years of age, had <10 pack-years of smoking history, and had all four grandparents self-

identified as Latino. Females were ineligible if they were in the third trimester of pregnancy. Asthma was defined as having both a history of physician diagnosed asthma and a self-reported presence of coughing, wheezing, or shortness of breath in the 2 years preceding enrollment. For this analysis, subjects were included based on availability of lung function parameters (outlined below), and PAI-1 genotyping for rs2227631, resulting in an analytic sample of 2070 subjects.

PAI-1 genotyping for rs2227631

Genome-wide genotyping was performed with the Axiom array as previously described.[25] The A allele within the SNP promoter site, rs2227631, for PAI-1 is a gain-of-function mutation that associates with higher PAI-1 plasma levels and is included in this analysis. In an exploratory analysis, we evaluated the individual associations of AG and AA genotypes on lung function (FEV_1, FVC, FEV_1/FVC ratio). Preliminary results indicated a correlation between the promoter mutation and lung function, particularly on the FEV_1/FVC ratio. The AG and AA groups were combined for primary analysis to increase precision of estimates even if these estimates would be more conservative. Notably, there was a clear dose effect for the A allele that remained statistically significant in our prior paper.[10] This was also noted in our current sensitivity analyses for the relative associations of the AA and AG genotypes on the FEV_1/FVC ratio (data not shown)

Lung function parameters

The lung function parameters of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), and the ratio of the two (FEV_1/FVC ratio) were measured at GALA II site visits for individuals with and without asthma. FEV_1/FVC ratio was the primary outcome measure given our prior finding of airway obstruction in asthmatic subjects. FEV_1 and FVC were secondary measures. Lung function testing was performed using a KoKo spirometer (nSpire Health, Longmont, CO) per the guidelines of the American Thoracic Society/ European Respiratory Society. Subjects required a minimum of three acceptable maneuvers to be included within analysis. References values for spirometry were determined using age, standing height (via calibrated stadiometer), sex, and ethnicity, using the National Health and Nutrition Examination Survey III reference standards.[26] Of the spirometry values for lung function, we analyzed percent predicted values of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and FEV_1/FVC ratio. Clinically relevant obstruction was defined as FEV_1/FVC ratio less than 0.75 (not predicted), which is the a pulmonary function cut point between mild and moderate to severe asthma in both adults and children in this age range (0.05 less than 0.80 as the lower limit of normal for this age range) according to the NAEPP guidelines.[27]

Exposures

Obesity – Study participants underwent weight and height measurements using standardized techniques and equipment during study recruitment. Body Mass Index (BMI) was calculated and categorized by percentile using standardized childhood sex- and age- specific growth charts (http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm). Subjects with a BMI<5^{th} percentile were classified as underweight, > 5^{th} and <85^{th} percentile were classified as healthy weight, the 85^{th}–94^{th} percentile were considered overweight, and ≥95th

percentile were classified as obese.[28] For the purposes of this analysis, subjects were classified as underweight, healthy weight, or overweight/obese.

Second hand smoke exposure – Parents reported maternal smoking in utero and during each of the following post-natal periods of life: under 2 years of age, and 3–6 years of life. We combined postnatal ages up to 6 years old to compare pre-natal and post-natal exposure in the analysis.

Lower respiratory tract viral illness in the first 2 years of life – This was based on parent report of a medical illness requiring medical attention in the first years of life.[10]

Replication cohort

The primary results were replicated in the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) cohort. This cohort contains individuals aged 12–56 who identify as African American, and have a physician diagnosis of asthma.[29] Data regarding the at-risk polymorphism, relevant covariates, and lung function was available for all included subjects.

Statistical analysis

Baseline characteristics of participants of asthmatic and non-asthmatic subjects included in this analytic sample were compared using *t* tests and chi-square tests. We first carried out linear regression on the population to determine the association of the at-risk genotype with lung function. We then tested the interaction term for genotype and asthma in the same model, retaining the primary variables for asthma and genotype. Following this, linear regression models were performed stratified by asthma status to determine the effect of the mutation on lung function parameters. All multivariate linear regressions included the following confounders: respiratory illness before age of two, household income, inhaled corticosteroid use, African and European ancestry, and site. Sex, ethnicity, and age are adjusted for within the percent predicted scores used within the analyses for lung function parameters, therefore they were not included as confounders in the linear regression model. Logistic regression analysis stratified by asthma status was used to determine if having the rs2227631 gain-of-function polymorphism was associated with increased risk of clinical obstruction. These analyses controlled for potential confounders including household income, inhaled corticosteroid use, African and European ancestry, and site. Again, sex, ethnicity, and age are incorporated within the percent predicted FEV_1/FVC ratio and were not included as confounders. Due to no subjects with the wild-type genotype having obstruction, we carried out a Fisher's exact test for obstruction in non-asthmatic subjects, but are not able to estimate an odds ratio.

To determine if key mediators (maternal obesity, pre- and postnatal smoke exposure, and respiratory illness prior to age two) mitigated any association between the at-risk genotype and lung function parameters, we included these serially and then in combination in the baseline linear regression model described above for lung function to determine change in magnitude and significance of associations. In all analyses, subjects with missing data for any included covariates were excluded.

Two secondary analyses were performed. We evaluated the association of the genotype with post-bronchodilator lung function by linear regression limited to asthmatic subjects as the bronchodilator response was not measured in controls. We also investigated the association between the at-risk-polymorphism and FEV_1/FVC ratio by age in subjects with asthma as a secondary analysis. Age was categorized as <12 or ≥12 years of age.

This study was approved by the institutional review boards at each study center (IRB for USCF, IRB for Northwestern University, IRB for The Office of Research Integrity and Complaince - The Ann and Robert H. Lurie Children's Hospital of Chicago (2008–13531), IRB for IRB for Texas Children's Hospital Baylor College of Medicine, IRB for Veteran's Caribbean Health Care System, IRB for Centro Neumologica Pediatrica, IRB for Jacobi Medical Center, IRB for CUNY). Written informed consent was obtained from the parents or legal guardians of all children and adult participants, and written informed assent was obtained from all children aged 12 – 18 years.

Results

Subject characteristics of the overall study sample and divided by those with and without asthma are presented in Table 1. The mean age of the study population was 13±3.45 years and majority of participants were Puerto Rican (40.1%) or Mexican (38.2%). There were no differences between asthmatic and control subjects in the distribution of the at-risk genotype. Similarly, there were no difference in SES, and smoke exposure during the post-natal period up to six years old. However, asthmatic subjects had more Puerto Rican and less Mexican subjects than non-asthmatic subjects, a greater proportion of African ancestry, and a greater proportion of subjects who were exposed to in-utero tobacco exposure than non-asthmatic subjects respectively. As expected, all lung function measures were lower in asthmatic subjects than non-asthmatic subjects (Table 1). Absolute measurements of pulmonary function in all subjects based on their asthma and genotype status are presented in Table E1.

Initial regression analysis of all subjects (asthmatic and non-asthmatic subjects combined) revealed no association of the rs2227631 gain-of-function genotype on the percent predicted FEV_1 or FVC (Table 2). The gain-of-function genotype (AA+AG) showed an inverse association with the FEV_1/FVC ratio with all subjects combined (β: −0.840; 95%CI: −1.628, −0.051). Within subjects with asthma, the genotype had an even greater magnitude of association with FEV_1/FVC ratio and a higher level of significance (β: −1.247; 95%CI: −2.142, −0.350). This association was not seen in subjects without asthma (n=378, Table 2). We also carried out a sensitivity analysis without combining the AG and AA genotypes and found a dose effect with a greater effect for AA (β: −1.50; 95%CI: −2.81, −0.19) that AG subjects (β: −1.17; 95%CI: −2.11, −0.22) compared to the GG genotype (control). We carried out an interaction analysis and found asthma status modified the effect of genotype on FEV_1/FVC ratio (p=0.03). We then carried out a joint analysis to quantify the observed effect. Compared to non-asthmatic subjects with the wild type genotype, the FEV_1/FVC ratio in those subjects with asthma with the at-risk polymorphism was decreased (β: −5.18; 95%CI: −6.72, −3.64). This decrease was greater in magnitude than that seen in asthma alone (β: −3.85; 95%CI: −5.41, −2.29). There was no effect due to the risk allele alone (β: 0.77; 95%CI: −0.97, 2.52).

To determine whether these associations were mitigated by other factors known to increase PAI-1 levels (second hand smoke exposure (pre- and postnatal), obesity, and pulmonary illnesses prior to two years old), we added these variables individually and then in combination to the base model. None of these associations individually or in combination significantly changed the effect size or significance of the association of the at-risk polymorphism on FEV_1/FVC (Table 3). As a sensitivity analysis to further account for chronic exposures that would increase PAI-1 levels, obesity, and pre- and postnatal smoke exposure, we limited the analyses to individuals who did not have these characteristics. When limited to non-obese asthmatic subjects, the association persisted (β: −1.53; 95%CI: −2.93, −0.14). Similarly, the association persisted in those who were not exposed to pre-natal (β: −1.48; 95%CI: −2.62, −0.34) and post-natal environmental tobacco smoke (β: −1.27; 95%CI: −2.21, −0.34).

We investigated the association of the at-risk genotype with pulmonary obstruction, defined as FEV_1/FVC ratio <0.75. In a non-stratified analysis, individuals with the at-risk genotype had an increased risk of clinical obstruction (OR: 1.376, 95%CI: 1.013 – 1.871, Table 4). When stratified by asthma status, those with the at-risk genotype and asthma retained a 39% increased odds of obstruction (OR: 1.394, 95%CI: 1.014 – 1.915, Table 4). There was no association between genotype and obstruction in subjects without asthma. (p=0.37, Fischer Exact test).

We carried out two secondary analyses. The age-stratified analysis in subjects with asthma suggests that the association of the gain-of-function polymorphism is seen primarily within children under 12 years of age (β = −1.822, CI: −3.191 – −0.453). The association of genotype with FEV1/FVC was not significant within older children in whom the association of obesity with FEV_1/FVC % predicted (β = −3.29, CI: CI: −4.57 – −2.01) was more pronounced than genotype. When evaluating post bronchodilator lung function, the findings for the FEV1/FVC ratio remained significant. However, there was a decreased effect size of the polymorphism on the FEV_1/FVC ratio within asthma subjects compared to controls without the at-risk genotype (β: −1.06, CI: −1.8112 – −0.310, p=0.006).

We replicated the main findings from the GALA II cohort within the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity (SAPPHIRE) cohort. We replicated the interaction between the at-risk polymorphism and asthma on FEV_1/FVC ratio (p = 0.028). In non-asthmatic subjects with the at-risk genotype (n=413) there was no difference in the percent predicted FEV_1/FVC ratio (β = 1.49, 95%CI: −0.03 – 3.01) compared to control subjects without the at-risk polymorphism. In contrast, subjects with both the at-risk phenotype and asthma had a significant decrease in FEV_1/FVC ratio (β = −1.49%; 95%CI −2.76 - −0.15). Finally, asthmatic subjects with <0.75 for the FEV_1/FVC ratio were 1.58 times (95%CI 1.07–2.35; p=0.022) more likely to be carriers of the risk allele in comparison to asthmatics with values ≥0.75 for FEV_1/FVC ratio.

Discussion

We recently demonstrated that a gain-of-function polymorphism in the PAI-1 promoter region leads to an increased risk of asthma and reduced lung function in asthmatic subjects

in the context of severe early life lower respiratory infection.[10] Our group, among others, has also found that PAI-1 is associated with altered lung function and increased odds of hospitalization/ER utilization in subjects with asthma.[10,30,31] However, based on the biological action of PAI-1 we expected to see that any genotype effect would be modified by asthma status since healthy controls would not have ongoing airway inflammation. Indeed, we found an interaction between asthma status and PAI-1 genotype on FEV_1/FVC ratio whereby the rs227631 gain-of-function mutation was associated with FEV_1/FVC ratio and airway obstruction in subjects with asthma, but not in healthy control subjects. Moreover, subjects with the at-risk polymorphism were at a 39% increased odds for airway obstruction (FEV_1/FVC <80% predicted) compared to asthmatic subjects without the at-risk genotype. This is relevant because a decreased FEV_1/FVC will alter a child's clinical care by modifying the category they are placed within stepped therapy guidelines.

While the majority of subjects had normal percent predicted pulmonary function, the genotype was associated with a decrease in percent predicted FEV_1/FVC ratio in both the pre- and post-bronchodilator analysis. The fact that the association persists in post bronchodilator lung function would suggest that any genotype associated airflow limitation has a fixed component. We also found that the effect is greater in younger children. However, our analysis suggests that the lack of significance for the effect in children 12 years of age an older may be due to an increasing effect of obesity (which also increases PAI-1) in this age group ($\beta = -3.29$ in children ≥12 years of age compared with −0.21 in children <12 years of age). Therefore, the effects of obesity may overshadow the independent effects of the at-risk polymorphism in the older age group. Based on these results we believe that the PAI-1 polymorphism serves as a risk-factor for airway obstruction in patients with asthma, particularly in children <12 years of age.

Our study is not able to determine causality or mechanism. However, there are different ways that PAI-1 may affect lung function. Coagulation and fibrin pathways are activated in non-exacerbated asthmatics, including higher concentration of PAI-1 in circulating blood.[32–34] Over-production of PAI-1, in cooperation with other coagulation factors activated at baseline in asthmatics, may move the hemostatic equilibrium to impaired fibrinolysis. Because the fibrinolytic system is important during repair processes, reduced fibrinolytic capacity may contribute to the impaired restoration of inflamed lung tissue. However, PAI-1 may also enhance airway inflammation by activation of G-protein coupled protease activated receptors (PAR's) expressed on smooth muscle and epithelial airway cells.[35] Activation of these may lead to an increase in inflammatory cytokine profile including interleukin-6 and platelet derived growth factor, furthering airway inflammation and airway structural change.[34] We are not able to differentiate whether these findings are secondary to systemic effects of PAI-1 itself or due to a localized effect due to increased airway expression. Thus, further work is needed to further elucidate the mechanism behind this outcome.

Plasminogen activator inhibitor 1 (PAI-1) is secreted by both airway epithelial cells[36] and human mast cells upon stimulation.[37] Plasminogen activator is essential in regulating fibrotic processes and is inhibited by the PAIs, in particular PAI-1.[13] In the airway, overexpression of PAI-1 leads to increased lung injury and deposition of extracellular

matrix.[13,14] Latino Americans have approximately 65% higher PAI-1 protein levels compared to African Americans and non-Latino Caucasians.[23,24] Therefore a gain-of-function polymorphism has even greater potential to impact lung function in Latino populations. However, these findings were replicated in the African American SAPPHIRE cohort, suggesting that these findings are not specific to Latinos, and potentially generalizable.

Furthermore, environmental factors common in underserved Latino populations, including obesity and tobacco exposure,[16–18,38] also play a key role in regulating PAI-1 levels.[15,19–22,39–43] Adipose tissue, particularly visceral adipose tissue, produces PAI-1 and obesity is associated with increased circulating PAI-1 levels.[15,21,39–41] Sputum PAI-1 levels increase in subjects with asthma during acute viral respiratory illnesses, including Influenza A viral infection leading to subsequent lung injury.[19,20] Smoking (passive and active) as well as nicotine exposure is associated with increased PAI-1 levels, particularly in individuals with PAI-1 gain-of-function mutations.[22,42,44,45] Since these PAI-1 promoting exposures are common, we expected them to drive much of the association of the gain-of-function mutation and lung function. We found that the PAI-1 polymorphism was still associated with airway obstruction after controlling these factors. This finding implies that ongoing low grade inflammation associated with asthma may be equally important in the effect of this polymorphism on lung function. This hypothesis may also have relevance for the finding of an interaction between genotype and asthma status.

There are several limitations to this study. Fewer subjects without asthma had pulmonary function available; however, there were no systematic differences in demographic features such as age, race, sex, and SES compared to controls without pulmonary function. Furthermore, all unstratified and stratified analyses controlled for key covariates. Also, while an in-depth analysis of non-asthmatics alone was not possible, determination of effect modification by asthma status was our primary objective and we were powered for this. Secondly, any individuals with the at-risk polymorphism, either heterozygotes or homozygotes, were combined for purposes of analysis to increase the stability of the estimates. In a secondary analysis, we carried out a sensitivity analysis with AA and AG separately and demonstrated a significant dose effect for both genotypes, making the combined estimate a conservative one. Both the SNP we looked at, rs2227631, and a insertion/ deletion variant in the PAI-1 promoter site (4G/5G), which is also associated with PAI-1 levels, are in strong linkage disequilibrium (D’=0.97).[46] We did not sequence the 4G/5G site, but the degree of LD would make it difficult to determine which of the two is functional even if we had done so. However, the s2227631 locus has been found to be the variant most closely associated with PAI-1 levels on GWAS,[47] and is used as a proxy for the 4G/5G site on arrays. Other limitations include that we did not evaluate mediation of genotype effect by other environmental factors, such as air pollution and allergen exposure. We had not focused on these factors as they are not described as major contributors to PAI-1 level. This will be an area for future study. The exposures of early life respiratory illness and maternal smoking were self-reported. While these exposures may be underreported due to reluctance to admit smoking in pregnancy or in the home, underreporting should not vary by genotype. Any misclassification would bias results toward the null hypothesis, making our findings even more robust. There were more females, slightly older ages, and slightly lower

levels of African ancestry in the healthy control group, however these differences are small in magnitude and were controlled for in our analysis both directly by ancestry variables and indirectly using percent predicted lung function. Lastly, this study was cross sectional and therefore we do not know how elevated PAI-1 levels affect lung function trajectory or if a critical window exists in early life where these levels are most influential.

In summary, we found that the association of a gain-of-function polymorphism in the PAI-1 promoter region, rs2227631, with lung function is modified by asthma status. The polymorphism is associated with decreased FEV_1/FVC ratio and increased prevalence of airway obstruction in Latino and African American subjects with asthma, but it has no effect in healthy controls. We further found that these associations persisted after accounting for common and chronic exposures such as smoking and obesity which increase PAI-1 levels. These findings suggest that this common genetic variant may be an important modifier of airway obstruction associated with asthmatic airway inflammation. Further studies should explore the trajectory of lung function change over time in older adults with the genotype and a longer duration of smoking and obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations (in order of appearance)

US	United States
SES	socioeconomic status
PAI-1	Plasminogen Activator Inhibitor-1
SNP	single nucleotide polymorphism
FEV_1	forced expiratory volume in 1 second
FVC	Forced Vital Capacity

GALA II Genes-environments and Admixture in Latino Americans

SAPPHIRE Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-ethnicity

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Table 1

Demographic and clinical characteristics of subjects

Variable	Total (n = 2070)	Asthma (n = 1692)	Non-asthma (n = 378)	*p*-value†
Age, mean (SD), years	13.0 (3.45)	12.6(3.3)	14.9(3.6)	**<0.001**
Male, No (%)	968(46.8)	752 (44.4)	216 (57.1)	**<0.001**
Recruitment center, No (%)				
IL	395 (19)	301	94	**<0.001**
TX	362 (17.5)	195	167	
NY	281 (13.6)	281	0	
SF	334 (16.1)	306	28	
PR	698 (33.7)	609	89	
Race				**<0.001**
Mexican	791 (38.2)	574	217	
Puerto Rican	831 (40.1)	727	104	
Other Latino	372 (18)	332	40	
Mixed Latino	76 (3.7)	59	17	
Ancestry proportion, mean (SD)				
African	0.13 (0.13)	0.14(0.13)	0.09 (0.09)	**<0.001**
European	0.51 (0.19)	0.52 (0.19)	0.47 (0.18)	**<0.001**
Current inhaled steroid, No (%)		602 (29.1)		
Respiratory illness before 2 years old No (%)	958 (46.9)	929 (55.4)	29 (7.9)	**<0.001**
Household income, No (%)				0.11
< $25,000	763 (36.7)	614 (36.3)	149 (39.4)	
$25–75,000	765 (37)	619 (36.6)	146 (38.6)	
> $75,000	542 (26.1)	459 (27.1)	83 (22)	
Mother smoke during pregnancy	104 (5.1)	96 (5.7)	8 (2.1)	**0.004**
Adults smoke postnatally (anytime up to 6 yrs)	556 (30.4)	478 (31.1)	78 (27)	0.17
rs2227631, No (%)				0.36
GG	810 (39.1)	655 (38.7)	155 (41)	

Variable		Total (n = 2070)	Asthma (n = 1692)	Non-asthma (n = 378)	*p*-value[†]
	AG	956 (46.2)	780 (46.1)	176 (46.7)	
	AA	304 (14.7)	257 (15.2)	47 (12.4)	
Lung function, mean (SD)					
FEV_1 % predicted (pre-BD)		92.3 (15.6)	90.9 (16.1)	98.4 (11.9)	**<0.001**
FVC % predicted (pre-BD)		95.9 (15.6)	95.4 (16.2)	97.9 (12.5)	**0.007**
FEV_1/FVC ratio % predicted (pre-BD)		96.5 (8.9)	95.5 (9.0)	100.8 (6.8)	**<0.001**

[†]P-values based on chi-square (categorical) and t-test (continuous) test for asthma compared with controls.

Table 2

Associations of the at-risk genotype (AA or AG) vs. control (GG) genotype on lung function parameters in all subjects and stratified by asthma status:

	GALA II			SAPPHIRE		
	B*	**95% Confidence Interval***	***p*-value†**	**B***	**95% Confidence Interval***	***p*-value†**
Total						
FEV_1 % predicted	−1.109	−2.437 – 0.220	0.10	−1.316	−3.13 – 0.50	0.155
FVC % predicted	−0.359	−1.681 – 0.962	0.59	−1.082	−2.73 – 0.57	0.198
FEV_1/FVC ratio % predicted	−0.840	−1.628 – 0.051	**0.04**	**−0.566**	**−1.65** – 0.52	**0.307**
Asthma						
FEV_1 % predicted	−1.165	−2.675 – 0.346	0.13	1.509	−3.65– 0.63	0.166
FVC % predicted	−0.058	−1.572 – 1.457	0.94	−0.416	−2.35– 1.52	0.674
FEV_1/FVC ratio % predicted	−1.247	−2.142 – −0.35	**0.006**	−1.46	−2.76 – −0.15	**0.029**
Controls						
FEV_1 % predicted	−1.235	−3.864 – 1.214	0.32	−1.110	−4.15 – 1.93	0.475
FVC % predicted	−1.505	−4.026 – 1.017	0.24	−2.855	−5.92 – 0.21	0.069
FEV_1/FVC ratio % predicted	0.381	−0.969 – 1.730	0.58	1.49	−0.03 – 3.01	0.056

*Adjusted for recruitment site location, household income, maternal education status, ancestry, inhaled corticosteroid use, (outcome variables are in percent predicted which incorporate age, sex, and race)

Table 3

Associations of key variables which increase PAI-1 levels on the association of the rs2227631 polymorphism and FEV_1/FVC ratio within asthmatic subjects

Variable	β for at risk genotype*	95%* Confidence Interval	*p*-value†
Base model			
	−1.247	−2.142 – −0.35	**0.006**
Models with PAI-1 increasing exposures			
Environmental tobacco smoke			
Maternal smoking during pregnancy	−1.303	−2.205 – −0.401	**0.005**
Exposure to ETS postnatally to age 6 yrs	−1.453	−2.403 – −0.502	**0.003**
Respiratory illness before 2 years old	−1.273	−2.177 – −0.370	**0.006**
Child obesity	−1.252	−2.143 – −0.361	**0.006**
Model with all PAI-1 increasing exposures included	−1.507	−2.464 – −0.551	**0.002**

*Adjusted for recruitment site location, household income, ancestry, inhaled corticosteroid use. (Outcome variables are in percent predicted which incorporate age, sex, and race.)

†*P*-values from linear regression analysis with individual incorporation of each variable to determine change in the magnitude and significance of associations

Table 4

Odds ratio of having clinical obstruction (FEV1/FVC ratio <0.75) for subjects with the at-risk genotype (AA/AG) of rs2227631 overall and stratified by asthma status.

	GALA II			SAPPHIRE		
	OR	**95% CI**	**p-value†**	**OR**	**95% CI**	**p-value†**
Unstratified model:						
Total	1.377	1.013 – 1.872	**0.041**	**1.492**	**1.03 – 2.17**	**0.036**
Model by asthma status:						
Asthma	1.394	1.014 – 1.915	**0.041**	**1.582**	**1.07 – 2.35**	**0.022**
Control	--	--	0.37	1.485	0.36 – 6.17	0.586

OR, odds ratio; CI, confidence interval

*Adjusted for recruitment site location, household income, pet/animal exposure under 2 years old, maternal education status, ancestry, inhaled corticosteroid use

†*P*-values for all subjects and asthmatic subjects from logistic regression analysis. P value for controls in GALA II by Fischer exact test.