

City University of New York (CUNY)

CUNY Academic Works

Publications and Research

Lehman College

2016

Association of a PAI-1 Gene Polymorphism and Early Life Infections with Asthma Risk, Exacerbations, and Reduced Lung Function

Seong H. Cho
Northwestern University

Jin-Young Min
Northwestern University

Dong Young Kim
Northwestern University

Sam S. Oh
University of California, San Francisco

Dara R. Torgerson
University of California, San Francisco

See next page for additional authors

[How does access to this work benefit you? Let us know!](#)

More information about this work at: https://academicworks.cuny.edu/le_pubs/221

Discover additional works at: <https://academicworks.cuny.edu>

This work is made publicly available by the City University of New York (CUNY).
Contact: AcademicWorks@cuny.edu

Authors

Seong H. Cho, Jin-Young Min, Dong Young Kim, Sam S. Oh, Dara R. Torgerson, Maria Pino-Yanes, Donglei Hu, Saunak Sen, Scott Huntsman, Celeste Eng, Harold J. Farber, William Rodriguez-Cintron, Jose R. Rodriguez-Santana, Denise Serebrisky, Shannon M. Thyne, Luisa N. Borrell, L. Keoki Williams, William DuPont, Max A. Seibold, Esteban G. Burchard, Pedro C. Avila, and Rajesh Kumar

RESEARCH ARTICLE

Association of a PAI-1 Gene Polymorphism and Early Life Infections with Asthma Risk, Exacerbations, and Reduced Lung Function

Seong H. Cho^{1,2}, Jin-Young Min³, Dong Young Kim¹, Sam S. Oh⁴, Dara R. Torgerson⁴, Maria Pino-Yanes⁴, Donglei Hu⁴, Saunak Sen⁵, Scott Huntsman⁴, Celeste Eng⁴, Harold J. Farber⁶, William Rodriguez-Cintron⁷, Jose R. Rodriguez-Santana⁸, Denise Serebrisky⁹, Shannon M. Thyne¹⁰, Luisa N. Borrell¹¹, L. Keoki Williams^{12,13}, William DuPont¹⁴, Max A. Seibold¹⁵, Esteban G. Burchard⁴, Pedro C. Avila¹, Rajesh Kumar^{16,17*}



OPEN ACCESS

Citation: Cho SH, Min J-Y, Kim DY, Oh SS, Torgerson DR, Pino-Yanes M, et al. (2016) Association of a PAI-1 Gene Polymorphism and Early Life Infections with Asthma Risk, Exacerbations, and Reduced Lung Function. PLoS ONE 11(8): e0157848. doi:10.1371/journal.pone.0157848

Editor: Kazuhiro Ito, National Heart and Lung Institute, UNITED KINGDOM

Received: March 2, 2016

Accepted: June 6, 2016

Published: August 24, 2016

Copyright: © 2016 Cho et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. Additional genetic files if required, are available from the dbGAP database (accession number phs000920.v1.p1).

Funding: Supported in part by the American Heart Association National Scientist Development Award, and by the National Institutes of Health (R01-ES015794, R01-HL088133, R01-HL078885, and R01-HL104608, R01—HL118267, R01-AI077439, R01-CA113710); National Institute On Minority Health And Health Disparities of the National Institutes of Health.

1 Division of Allergy-Immunology, Department of Medicine, Northwestern University, Chicago, Illinois, United States of America, **2** Division of Allergy-Immunology, Department of Internal Medicine, University of South Florida, Tampa, Florida, United States of America, **3** Department of Otolaryngology, Northwestern University, Chicago, Illinois, United States of America, **4** Department of Medicine, University of California, San Francisco, California, United States of America, **5** Division of Biostatistics, Department of Preventive Medicine, UTHSC, Memphis, Tennessee, United States of America, **6** Department of Pediatrics, Section of Pulmonology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas, United States of America, **7** Veterans Caribbean Health Care System, San Juan, Puerto Rico, United States of America, **8** Centro de Neumologia Pediatrica, CSP, San Juan, Puerto Rico, United States of America, **9** Pediatric Pulmonary Division, Jacobi Medical Center, Bronx, New York, United States of America, **10** Department of Pediatrics, University of California, San Francisco, California, United States of America, **11** Department of Health Sciences, Lehman College, CUNY, New York, United States of America, **12** Department of Internal Medicine, Henry Ford Health System, Detroit, Michigan, United States of America, **13** Center for Health Policy and Health Services Research, Henry Ford Health System, Detroit, Michigan, United States of America, **14** Department of Biostatistics, Vanderbilt University Medical School, Nashville, Tennessee, United States of America, **15** Center for Genes, Environment and Health, National Jewish Health, Denver, Colorado, United States of America, **16** Division of Allergy-Immunology, Department of Pediatrics, Northwestern University, Chicago, Illinois, United States of America, **17** The Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, United States of America

☯ These authors contributed equally to this work.

* rkumar@luriechildrens.org

Abstract

Background

Plasminogen activator inhibitor-1 (PAI-1) is induced in airways by virus and may mediate asthmatic airway remodeling. We sought to evaluate if genetic variants and early life lower respiratory infections jointly affect asthma risk.

Methods

We included Latino children, adolescents, and young adults aged 8–21 years (1736 subjects with physician-diagnosed asthma and 1747 healthy controls) from five U.S. centers and Puerto Rico after excluding subjects with incomplete clinical or genetic data. We evaluated the independent and joint effects of a *PAI-1* gain of function polymorphism and bronchiolitis / Respiratory Syncytial Virus (RSV) or other lower respiratory infections (LRI) within the first 2 years of life on asthma risk, asthma exacerbations and lung function.

Health under Award Number P60-MD006902; M01-RR00188 to H.J.F.; the Flight Attendant Medical Research Institute (FAMRI), the Sandler Foundation, the RWJF Amos Medical Faculty Development Award (to E.G.B.), the American Asthma Foundation (to E. G.B.); Ernest S. Bazley Grant (to PCA); General Clinical Research Center (to HF).

Competing Interests: The authors have declared that no competing interests exist.

Abbreviations: (In order of appearance): US, United States; PAI-1, Plasminogen Activator Inhibitor-1; ECM, extracellular matrix; SNP, single nucleotide polymorphism; FEV₁, forced expiratory volume in 1 second; LRI, lower respiratory tract infection; RSV, respiratory syncytial virus; GALA II, Genes-environments and Admixture in Latino Americans; GAGE II, Study of African Americans, Asthma, Genes & Environments II; FVC, Forced Vital Capacity.

Results

RSV infection (OR 9.9, 95%CI 4.9–20.2) and other LRI (OR 9.1, 95%CI 7.2–11.5) were independently associated with asthma, but *PAI-1* genotype was not. There were joint effects on asthma risk for both genotype-RSV (OR 17.7, 95% CI 6.3–50.2) and genotype-LRI (OR 11.7, 95% CI 8.8–16.4). A joint effect of genotype-RSV resulted in a 3.1-fold increased risk for recurrent asthma hospitalizations. In genotype-respiratory infection joint effect analysis, FEV₁% predicted and FEV₁/FVC % predicted were further reduced in the genotype-LRI group (β -2.1, 95% CI -4.0 to -0.2; β -2.0, 95% CI -3.1 to -0.8 respectively). Similarly, lower FEV₁% predicted was noted in genotype-RSV group (β -3.1, 95% CI -6.1 to -0.2) with a trend for lower FEV₁/FVC % predicted.

Conclusions

A genetic variant of *PAI-1* together with early life LRI such as RSV bronchiolitis is associated with an increased risk of asthma, morbidity, and reduced lung function in this Latino population.

Introduction

Asthma affects more than 25 million people in the United States including 9.3% of all US children, with \$56 billion in annual healthcare and indirect costs.[1] Studies have suggested a range of 47 to 95% heritability for asthma,[2–4] with multiple associated genetic variants.[5] However, the individual effects of these variants are small,[6,7] leading to questions about whether these genetic influences are more relevant in the broader context of specific environmental exposures.[8] Among the early environmental exposures associated with asthma, viral respiratory infections are among the most important.[9,10] While interaction between viral illness and genetic variants on asthma risk has been reported,[11] mechanisms remain unclear.

A gene-infection interaction promoting airway remodeling and lung function decline may be important both in asthma and generating severe asthma phenotypes. The plasmin and fibrinolytic pathway may be particularly relevant in airway remodeling and upper respiratory infection is associated with increased fibrinogenic activities in subjects with recurrent wheezing or asthma.[12,13] Specifically, *plasminogen activator inhibitor-1 (PAI-1)* promotes fibrosis,[14,15] and blocking of this enzyme prevents extracellular matrix (ECM) deposition.[16,17] URI increases airway *PAI-1* levels, with virus inducing *PAI-1* production in human airway epithelial cells.[12] *PAI-1* promoter site genetic variants are strongly associated not only with plasma *PAI-1* levels,[18] but also with increased risk of asthma, decreased forced expiratory volume in 1 second (FEV₁), and airway hyperreactivity.[19] [20]

We utilized the Genes-environments and Admixture in Latino Americans (GALA II) study to test our hypothesis that a *PAI-1* polymorphism in combination with infection in early life may be associated with asthma, asthma severity, and worse lung function.

Materials and Methods

Recruitment

Latino children, adolescents, and young adults aged 8–21 years from five centers (Chicago, Illinois; Bronx, New York; Houston, Texas; San Francisco Bay Area, California; and Puerto Rico)

were enrolled in the GALA II cohort study from 2006 to 2011 ($n = 4157$ children of whom 2022 had asthma and 2135 were healthy controls). We excluded subjects who had incomplete genetic or clinical data for relevant covariates ($n = 286$ asthmatics and 388 controls), yielding an analyzing sample size of 3483 subjects (1736 with asthma and 1747 without asthma). Further details are available on the online supporting information file.

PAI-1 genotyping for *rs2227631*

Genome-wide genotyping was performed with the Axiom LAT1 array (World Array 4; Affymetrix, Santa Clara, Calif) as previously described.[21] The A allele of the promoter site SNP, *rs2227631*, for the PAI-1 gene is a gain of function mutation associated with higher plasma levels of PAI-1,[18] and is included in this chip. In initial exploratory analyses, we evaluated the individual effects of AG and AA genotypes in combination with infection on asthma risk. While there was a dose effect, numbers were not sufficiently large to separately analyze the AA-infection group (for example AA-RSV is only 0.9% (31/3446)). The AG and AA groups were combined for the primary analysis. For completeness, a secondary analysis for the primary outcome of asthma is presented in the E-tables with genotype expressed as GG, AG, and AA.

Bronchiolitis / RSV

Symptomatic bronchiolitis / RSV episodes requiring medical attention within the first 2 years of life was ascertained by the following question: "Was <CHILD> diagnosed with bronchiolitis or RSV before age 2 yrs."

Other lower respiratory illness

Symptomatic lower respiratory tract illnesses (LRI) requiring medical attention within the first 2 years of life was ascertained by the following question: "Was <CHILD> seen by a doctor for chest illness before age 2 yrs."

Subject exposure classification

Subjects were divided into subgroups to identify the independent and joint effects of LRI requiring medical attention and *rs2227631* genotype as follows: GG without LRI (GG-No LRI), AG/AA without any history of LRI (AG/AA No LRI), GG with history of LRI (GG+LRI), and AG/AA with history of LRI (AG/AA+LRI). A similar grouping was carried out for genotype and symptomatic bronchiolitis / RSV, with "no RSV" indicating that there was no severe symptomatic RSV bronchiolitis requiring medical attention (i.e. no severe RSV).

Outcome measures

The primary outcome was report of physician diagnosed asthma. Secondary outcomes included asthma exacerbations and lung function. Spirometry was performed using a KoKo spirometer (nSpire Health, Longmont, CO) according to the guidelines of the American Thoracic Society/European Respiratory Society. Measures analyzed included percent predicted FEV1, FVC, and FEV1/FVC ratio (National Health and Nutrition Examination Survey III reference standards). Subjects with asthma were categorized for exacerbation status based on each the following criteria assessed over the previous 12 months: oral steroid use for 2 or more weeks, ≥ 2 emergency room (ER) visits, and ≥ 2 hospital admissions.

Replication cohort

The Study of African Americans, Asthma, Genes & Environments II (SAGE II) was used to replicate the associations found in the GALA II study and is described elsewhere.[22] Briefly, the SAGE II study is an ongoing clinic-based multicenter asthma case-control study, including African American subjects with asthma (n = 666) recruited from the San Francisco bay area, conducted in parallel to GALA II using identical protocols and questionnaires.

Statistical analysis

For descriptive statistics, the study population was divided into asthma and control subjects, and χ^2 tests and *t* tests performed to describe differences in terms of demographic and clinical characteristics. Analyses on the outcome of asthma diagnosis included all subjects. However, asthma exacerbations and lung function analyses were limited to subjects with asthma. Separate logistic regression analyses were performed to estimate the associations of asthma with the *PAI-1* genotype at the *rs2227631* locus, early-infection (RSV or LRI) history, and the joint effect of genotype and infection. Similar logistic regression analyses were carried out for the outcome of asthma exacerbations limited to subjects with asthma. Both analyses controlled for age, sex, ethnicity, ancestry, socioeconomic factors, environmental factors (history of farm animal exposure, received antibiotics during first year), family history of asthma, African and European genetic ancestry, and recruitment site. For the analysis of lung function (percent predicted values) within asthmatics we used a multivariate linear regression model, adjusting for age, sex, ethnicity, socioeconomic factors, environmental factors (history of smoke exposure), medication for asthma control, and recruitment site. Lung function analyses which included the SNP or SNP-infection analyses also controlled for ancestry. For the asthma analyses, separate models were also generated to test for the significance of interaction terms for infection and risk genotype, including terms for the main effects of genotype risk allele and infection history.

Replication of these analyses was carried out in the SAGE study using the same approach. All statistical analyses were performed using SPSS software (Version 22.0, Statistical Package for Social Science, Chicago, IL, USA). P values < 0.05 were considered statistically significant.

This study was approved by the institutional review boards at each study center (IRB for USCF, IRB for Northwestern University, IRB for The Ann and Robert H. Lurie Children's Hospital of Chicago, 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.

Data used in the performance of this analysis is included with the paper in [S1 File](#).

Results

Baseline characteristics

[Table 1](#) presents the distribution of selected characteristics for the overall study sample divided by asthma status. The mean age of the study population was 13 years and majority of participants were Mexicans (36.5%) or Puerto Rican (42.6%). Compared to those without asthma, subjects with asthma were more likely to have had a symptomatic episode of RSV bronchiolitis or other symptomatic lower respiratory illness before age 2 years old (9.3% vs 0.9%; or 55.4% vs 10.1%, respectively), but there was no significant difference in *PAI-1* genotype. Other differences by asthma status were minor in magnitude and are described in the online supplement.

Table 1. Demographic and clinical characteristics of subjects.

Variable	Total (n = 3483)	Asthma (n = 1736)	Non-asthma (n = 1747)	P value
Age, mean (SD), years	13.18 (3.49)	12.60 (3.32)	13.75 (3.56)	0.000 ^a
Male, No (%)	1735 (49.8)	961 (55.4)	774 (44.3)	0.000 ^b
BMI, mean (SD)	23.51 (6.66)	23.25 (6.56)	24.60 (6.93)	0.000 ^a
Ethnicity, No (%)				
Mexican	1271 (36.5)	602 (34.7)	669 (38.3)	0.027 ^b
Puerto Rican	1485 (42.6)	735 (42.3)	750 (42.9)	0.724
Other Latino	620 (17.8)	338 (19.5)	282 (16.1)	0.010 ^b
Mixed Latino	106 (3.0)	61 (3.5)	45 (2.6)	0.107
Ancestry proportion, mean (SD)				
African	0.14 (0.13)	0.15 (0.13)	0.13 (0.12)	0.000 ^a
European	0.59 (3.81)	0.54 (0.19)	0.65 (5.37)	0.216
Country born: USA, No (%)	1703 (49.6)	732 (43.0)	971 (56.2)	0.000 ^b
Recruited center, No (%)				
IL	639 (18.4)	310 (17.9)	329 (18.8)	0.492
TX	365 (10.5)	197 (11.4)	168 (9.6)	0.087
NY	545 (15.7)	290 (16.8)	255 (14.6)	0.078
SF	636 (18.3)	317 (18.3)	319 (18.3)	0.955
PR	1289 (37.1)	614 (35.5)	675 (38.7)	0.056
Frequency of allergic sensitization	1809 (52)	1076 (62)	733 (42)	
Family history of Asthma, No (%)				
Mother	771 (22.8)	561 (33.1)	210 (12.4)	0.000 ^b
Father	463 (14.4)	334 (20.8)	129 (8.0)	0.000 ^b
Siblings	1343 (41.6)	866 (53.4)	477 (29.8)	0.000 ^b
Infection history before age 2 yrs, No (%)				
RSV bronchiolitis	177 (5.1)	161 (9.3)	16 (0.9)	0.000 ^b
LRI	1126 (32.7)	952 (55.4)	174 (10.1)	0.000 ^b
Environments				
Pet exposure during 1 st yr of life, No (%)				
Cat	371 (10.8)	179 (10.4)	192 (11.1)	0.501
Dog	1165 (33.8)	542 (31.4)	623 (36.1)	0.003 ^b
Farm animals	358 (10.4)	148 (8.6)	210 (12.1)	0.001 ^b
Smoke, No (%)				
Mother smoke during pregnancy	168 (4.9)	97 (5.6)	71 (4.1)	0.039 ^b
Adults smoke before age 2yrs	784 (25.3)	437 (27.7)	347 (22.8)	0.002 ^b
Children ever tried smoking	150 (4.3)	58 (3.3)	92 (5.3)	0.005 ^b
Children current smoking	1 (0.1)	0 (0.0)	1 (0.2)	0.450
Received antibiotics during 1 st yr of life, No (%)	1288 (41.7)	774 (49.7)	514 (33.6)	0.000 ^b
Daycare, No (%)	812 (23.8)	443 (25.9)	369 (21.7)	0.004 ^b
Socioeconomic characteristics				
Mother education, No (%)				
< High school	1356 (38.9)	656 (37.8)	700 (40.1)	0.168
High school	913 (26.2)	471 (27.1)	442 (25.3)	0.219
Some college	1214 (34.9)	609 (35.1)	605 (34.6)	0.781
Income, No (%)				
< \$25,000	1227 (35.2)	623 (35.9)	604 (34.6)	0.417
\$25–75,000	1195 (34.3)	634 (36.5)	561 (32.1)	0.006 ^b
> \$75,000	1061 (30.5)	479 (27.6)	582 (33.3)	0.000 ^b

(Continued)

Table 1. (Continued)

Variable	Total (n = 3483)	Asthma (n = 1736)	Non-asthma (n = 1747)	P value
Insurance, No (%)	2195 (93.1)	1631 (95.2)	1564 (91.0)	0.000 ^b
rs2227631, No (%)				
GG	1405 (40.3)	676 (38.9)	729 (41.7)	0.093
AG	1583 (45.4)	801 (46.2)	782 (44.8)	0.414
AA	495 (14.3)	259 (14.9)	236 (13.5)	0.233
AG+AA	2078 (59.7)	1060 (61.1)	1018 (58.3)	0.093
Lung function, mean (SD)				
FEV ₁ % predicted (pre-BD)	92.45 (15.65)	91.03 (16.03)	98.65 (12.04)	0.000 ^a
FVC % predicted (pre-BD)	96.03 (15.62)	95.55 (16.17)	98.14 (12.78)	0.000 ^a
FEV ₁ /FVC ratio % predicted (pre-BD)	96.53 (8.86)	95.54 (9.00)	100.86 (6.70)	0.000 ^a

BMI, body mass index; IL, Illinois; TX, Texas; NY, New York; SF, San Francisco; PR, Puerto Rico; RSV, respiratory syncytial virus; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; BD, bronchodilator

^a P < 0.05 from the t test for Asthma vs Non-Asthma.

^b P < 0.05 from the χ² test for Asthma vs Non-Asthma.

doi:10.1371/journal.pone.0157848.t001

Risk of asthma

Table 2 presents 2 separate models evaluating the effect of genotype, and infection (tested separately). Firstly, the PAI-1 SNP rs2227631 itself does not increase the odds of developing asthma. Second, there was a significant increase in the likelihood of asthma in subjects with a history of early life infection such as RSV bronchiolitis and other LRI (OR 9.9, 95% CI 4.9–20.2; OR 9.1, 95% CI 7.2–11.5, respectively). If the presence of either RSV or LRI was tested in a similar model, the findings were unchanged (OR 10.1, 95% CI 8.2–12.4). In Figs 1 and 2, we display the joint association of the PAI-1 risk genotype and early life infection with the diagnosis of asthma. While there was an increase of asthma risk in GG-RSV group, there was an even more dramatic increase in the AG/AA+RSV group (Fig 1—OR 4.1, 95% CI 1.5–11.2; OR 17.7, 95% CI 6.3–50.2, respectively). Similar findings of lesser magnitude were noted in the GG+LRI group and AG/AA+LRI group when compared to GG no LRI group (Fig 2—OR 7.7, 95% CI 5.5–11.0; OR 11.7, 95% CI 8.8–16.4, respectively). In models testing the interaction terms for LRI-genotype and RSV-genotype (accounting for main effects), the LRI-genotype interaction term was significant at a level of P = 0.014 and the RSV-genotype approached significance at a

Table 2. Adjusted effects of rs2227631, and infection on asthma.

Analyses	N	OR* (95% CI)	P value†
Genotype alone—rs2227631			
GG	1405	Reference	
AG/AA	2078	1.051 (0.878–1.259)	0.588
Infection history before age 2 yrs alone			
RSV bronchiolitis	177	9.920 (4.881–20.159)	0.000
LRI	1126	9.110 (7.233–11.474)	0.000

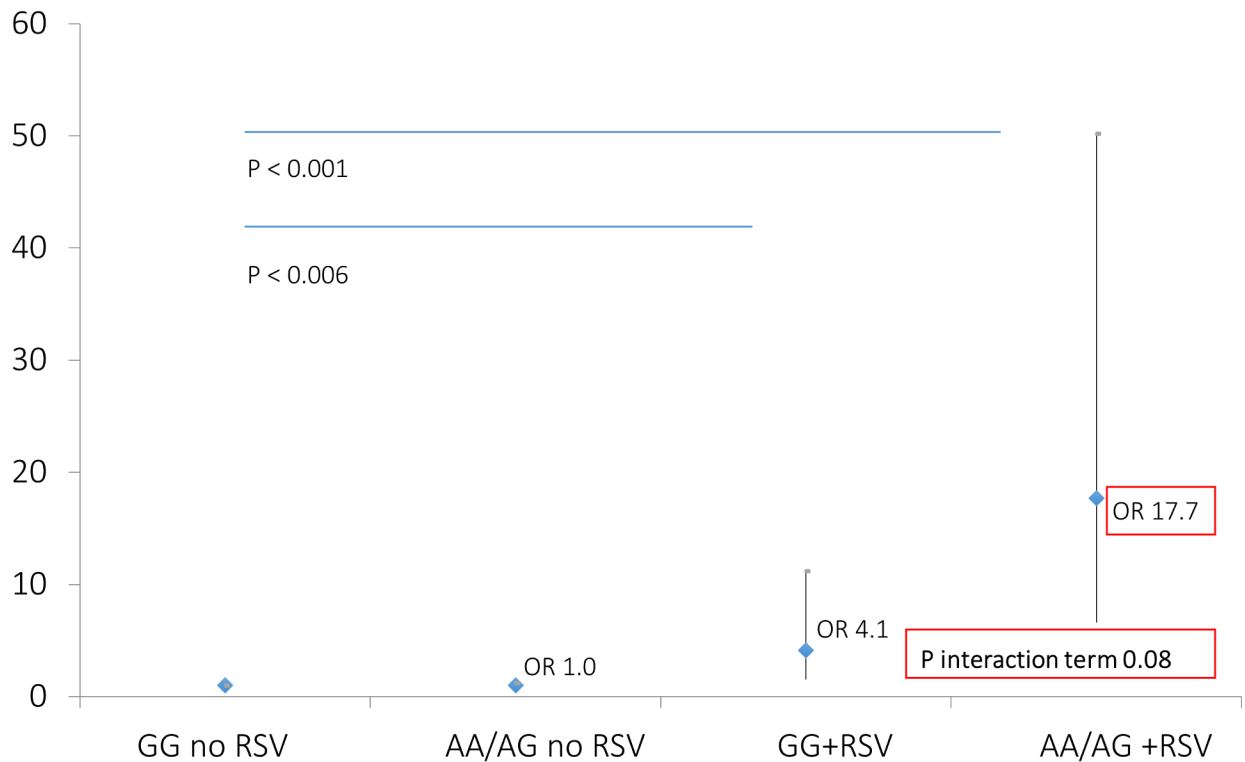
OR, odds ratio; CI, confidence interval; RSV, respiratory syncytial virus

*Adjusted for age, sex, ethnicity, ancestry, socioeconomic factors, environmental factors (history of farm animal exposure and received antibiotics during first year), family history of asthma and recruitment site.

†P values from multivariate regression analysis. Statistically significant P values are indicated in bold.

doi:10.1371/journal.pone.0157848.t002

Odds of Asthma by “RSV” and Genotype



*Adjusted for age, sex, ethnicity, ancestry, socioeconomic factors, environmental factors (history of farm animal exposure and received antibiotics during first year), family history of asthma and recruitment site.

Fig 1. Joint association of bronchiolitis/ RSV and PAI-1 risk genotype (rs2227631) with Asthma. RSV indicates subjects who were diagnosed with bronchiolitis or RSV before age 2 years old. Adjusted for age, sex, ethnicity, ancestry, socioeconomic factors, environmental factors (history of farm animal exposure and received antibiotics during first year), family history of asthma and recruitment site.

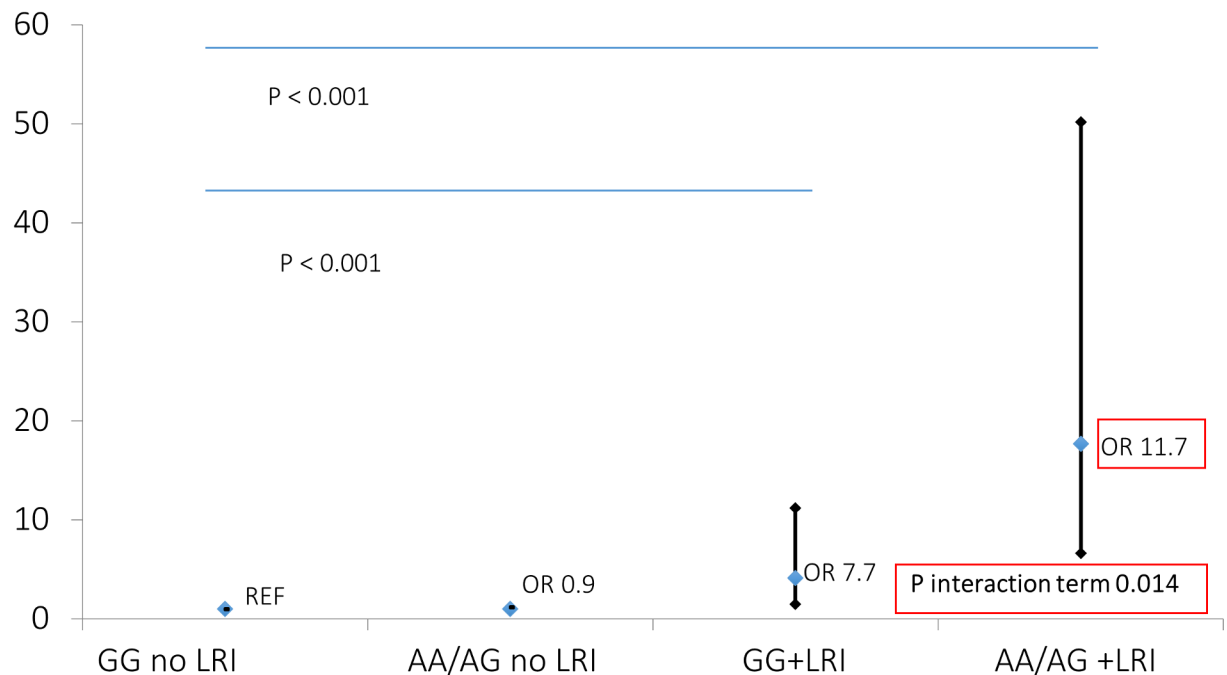
doi:10.1371/journal.pone.0157848.g001

level of $p = 0.08$. Furthermore, in a subgroup analysis limited to subjects who had RSV or LRI, there was a clear genotype effect for the risk allele (AG/AA+RSV OR 4.3, 95% CI 1.04–17.95; AG/AA+LRI OR 1.58, 95% CI 1.06–2.34) compared to those who had RSV or LRI with the wild type GG allele. Finally, we also carried out a sensitivity analysis (since RSV was by report) whereby we combined those with either RSV or LRI by these definitions and found that the magnitude of effect was similar for the AA/AG+ either LRI or RSV (OR 12.9, 95% CI 9.6–17.3). The interaction term for any lower respiratory infection and genotype (accounting for main effects) was significant at the $P = 0.002$ level.

Asthma Exacerbations

Table 3 presents 3 separate models (limited to subjects with asthma) evaluating the effect of genotype, infection, and then the joint effect of genotype and infection on asthma severity using three different parameters: steroid use ≥ 2 weeks, ER visit ≥ 2 times, and hospitalization ≥ 2 times all in the previous 12 months. Considering the effect of genotype alone, the AG/AA genotype showed more than 2 times higher risk of hospitalization ($P = 0.046$, OR 2.2 95% CI 1.0–4.6). Similarly, subjects with a history of RSV bronchiolitis had

Odds of Asthma by LRI/genotype



*Adjusted for age, sex, ethnicity, ancestry, socioeconomic factors, environmental factors (history of farm animal exposure and received antibiotics during first year), family history of asthma and recruitment site.

Fig 2. Joint association of early life lower respiratory tract infection and PAI-1 risk genotype (rs2227631) with Asthma. LRI indicates subjects who were diagnosed with a lower respiratory tract infection requiring medical attention before age 2 years old. Adjusted for age, sex, ethnicity, ancestry, socioeconomic factors, environmental factors (history of farm animal exposure and received antibiotics during first year), family history of asthma and recruitment site.

doi:10.1371/journal.pone.0157848.g002

significantly higher risk of having an ER visit ≥ 2 times in the previous 12 months ($P = 0.001$, OR 2.0 CI 95% 1.3–3.1). While findings were similar in direction for LRI, they failed to reach significance ($P = 0.18$, OR 1.2 95% CI 0.92–1.6, respectively). Finally, the PAI-1 SNP and RSV jointly increased the risk of ED visits and the risk of hospitalization by 1.8 and 3.1-fold respectively. However, there was no joint effect between the PAI-1 SNP and LRI on these asthma severity parameters.

Lung function

Table 4 presents 3 separate models (limited to subjects with asthma) evaluating the effect of genotype, infection, and then the joint effect of genotype and infection on lung function including three parameters; FEV₁% predicted, FVC % predicted, and FEV₁/FVC % predicted. Only the FEV₁/FVC ratio was reduced in AG/AA genotype compared to GG genotype. Early life history of LRI was associated with decreases in FEV₁/FVC ratio; but this was not significant for RSV bronchiolitis. When we looked at the joint effect of PAI-1 genotype-and early life infections, FEV₁% predicted, and FEV₁/FVC % predicted were further reduced in AG/AA+LRI group compared to GG no LRI group ($P = 0.03$, coefficient β -2.06 95% CI -3.97--0.16; and $P = 0.001$, coefficient β -1.97 95% CI -3.10--0.84, respectively). Similar findings were noted in

Table 3. Adjusted effect of rs2227631, infection and rs2227631 gene-infection interaction on asthma exacerbations.

Analyses	Steroid use ≥ 2 weeks in past 12months		ER visit ≥ 2 times in past 12months		Hospitalizations ≥ 2 times in past 12months	
	OR* (95%CI)	P†	OR* (95%CI)	P†	OR* (95%CI)	P†
Genotype alone—rs2227631						
GG	Reference		Reference		Reference	
AG/AA	1.078 (0.774–1.501)	0.66	0.931 (0.713–1.216)	0.60	2.152 (1.013–4.572)	0.046
Infection history before age 2 yrs alone						
RSV bronchiolitis	1.243 (0.800–1.931)	0.33	2.004 (1.314–3.057)	0.001	1.589 (0.740–3.412)	0.23
LRI	1.262 (0.910–1.752)	0.16	1.197 (0.917–1.562)	0.18	1.285 (0.670–2.464)	0.48
Gene-Infection analyses						
rs2227631-RSV						
GG No RSV	Reference		Reference		Reference	
AG/AA No RSV	1.089 (0.765–1.550)	0.63	0.911 (0.689–1.204)	0.51	1.728 (0.794–3.761)	0.17
GG+RSV	1.255 (0.529–2.977)	0.61	2.408 (1.053–5.506)	0.04	‡	0.99
AG/AA+RSV	1.274 (0.734–2.211)	0.39	1.854 (1.111–3.092)	0.02	3.107 (1.143–8.450)	0.03
rs2227631- LRI						
GG No LRI	Reference		Reference		Reference	
AG/AA No LRI	0.954 (0.547–1.664)	0.87	0.709 (0.469–1.069)	0.10	2.075 (0.566–7.602)	0.27
GG+LRI	1.115 (0.634–1.961)	0.71	0.859 (0.561–1.315)	0.49	1.195 (0.278–4.970)	0.80
AG/AA+ LRI	1.278 (0.758–2.155)	0.36	0.991 (0.668–1.472)	0.96	2.645 (0.750–9.334)	0.13

OR, odds ratio; CI, confidence interval; RSV, respiratory syncytial virus

*Adjusted for age, sex, ethnicity, socioeconomic factors, environmental factors (history of smoke exposure), medication for asthma control and recruitment site. Analyses of SNP and SNP-infection also controlled for ancestry.

†P values from multivariate regression analysis. Statistically significant P values are indicated in bold.

‡ unable to estimate stable odds ratio due to small cell size.

doi:10.1371/journal.pone.0157848.t003

the analyses of RSV with lower FEV₁% predicted in AG/AA-RSV group. The findings for FEV₁/FVC % predicted neared significance but showed a similar magnitude and direction of findings.

Replication

We evaluated the asthma associations in the SAGE cohort with similar results. The SNP itself was not associated with asthma (OR 1.27, 95% CI 0.85–1.88), while both RSV (OR 14.3, 95% CI 1.71–119.53) and LRI (OR 22.1, 95% CI 11.8–41.4) were associated. We also replicated the joint effects for SNP-LRI association. While there was an increase of asthma risk in GG-LRI group, there was an even more dramatic increase in the AG/AA+LRI group (OR 20.4, 95% CI 8.9–46.9; OR 26.7, 95% CI 11.2–63.9, respectively). Despite a similar direction and magnitude of association, we were not able to replicate the SNP-RSV associations in the smaller SAGE cohort due to small numbers (n = 9) in this sub-group (OR 5.81, 95%CI 0.54–62.3 for AA/AG +RSV group). We also were not able to replicate the exacerbation and lung function associations in SAGE, possibly due to the smaller sample size.

Discussion

This study examines the effect of a common polymorphism in the PAI-1 gene and early life lower respiratory infections including RSV/bronchiolitis in patients with asthma. While the genotype itself was not associated with asthma risk, there was a significant interaction between

Table 4. Adjusted effect of rs2227631, infection and rs2227631 gene-infection interaction on lung function in asthmatic subjects.

Analyses	FEV ₁ % predicted		FVC % predicted		FEV ₁ /FVC ratio % predicted	
	Coefficient β* (95% CI)	P [†]	Coefficient β* (95% CI)	P [†]	Coefficient β* (95% CI)	P [†]
Genotype alone—rs2227631						
GG	Reference		Reference		Reference	
AG/AA	-1.212 (-2.530–0.105)	0.07	-0.517 (-1.812–0.778)	0.43	-0.787 (-1.570 –0.003)	0.049
Infection history before age 2 yrs alone						
RSV bronchiolitis	-1.854 (-4.315–0.606)	0.14	-1.032 (-3.458–1.393)	0.40	-0.988 (-2.430–0.453)	0.18
LRI	-1.195 (-2.528–0.138)	0.08	-0.060 (-1.256–1.370)	0.93	-1.311 (-2.090 –0.532)	0.001
Gene-infection analyses						
rs2227631-RSV						
GG No RSV	Reference		Reference		Reference	
AG/AA No RSV	-1.051 (-2.414–0.312)	0.13	-0.299 (-1.639–1.041)	0.66	-0.844 (-1.655 –0.338)	0.04
GG+RSV	0.303 (-3.794–4.400)	0.88	1.005 (-3.024–5.033)	0.62	-0.848 (-3.285–1.589)	0.49
AG/AA+RSV	-3.113 (-6.050 –0.177)	0.04	-1.741 (-4.628–1.146)	0.23	-1.601 (-3.347–0.146)	0.07
rs2227631-LRI						
GG No LRI	Reference		Reference		Reference	
AG/AA No LRI	-0.757 (-2.546–1.032)	0.41	-1.000 (-2.759–0.758)	0.26	0.243 (-0.816–1.301)	0.65
GG+LRI	-0.326 (-2.382–1.731)	0.75	-0.348 (-2.369–1.673)	0.74	-0.095 (-1.122–1.311)	0.88
AG/AA+ LRI	-2.063 (-3.971 –0.155)	0.03	-0.234 (-2.109–1.641)	0.81	-1.971 (-3.101 –0.843)	0.001

FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; BD, bronchodilator; CI, confidence interval; RSV, respiratory syncytial virus

*Adjusted for BMI, socioeconomic factors, medication for asthma control and recruitment site. Lung function parameters (% predicted values) were already adjusted with age, sex, and race. Additionally genotype and genotype-infection analyses were corrected for ancestry.

†P values from multivariate regression analysis. Statistically significant P values are indicated in bold.

doi:10.1371/journal.pone.0157848.t004

early life infection and genotype on the outcome of asthma diagnosis. Asthma risk increased 17-fold when this genotype was present in individuals with symptomatic RSV / bronchiolitis infection and almost 12-fold in those with other LRI in early life requiring medical attention. This finding was replicated in the SAGE II cohort for LRI, but not RSV potentially due to the small numbers of SAGE II subjects in the AG/AA+RSV group. Both LRI and RSV infection severe enough to require medical attention (10% and 1% of our control subjects respectively) and the genotype in question are common.[23,24] The frequencies of AG and AA genotypes were 45.4% and 14.3%; with an overall A allele frequency of 37%.

The joint effect of early life lower respiratory tract infection and the gain of function PAI-1 SNP is in keeping with other studies which suggest that the PAI-1 pathway is important in airway response to virus, and that an exaggerated response may be detrimental. PAI-1 plasma levels are increased in young children who had history of URI-induced repeated wheeze.[13] Human rhinovirus infection increases the production of PAI-1 in primary airway epithelial cells from subjects with asthma, and during an URI in subjects with asthma, nasal lavage and sputum PAI-1 levels increase.[12] These findings serve as *in vitro* and *in vivo* evidence of impact of respiratory viral infections on PAI-1 production in asthma. In murine models, PAI-1 deficiency protected against airway fibrosis, whereas PAI-1 overexpression enhanced fibrotic changes.[14,15] Blocking PAI-1 using either siRNA for PAI-1 or a PAI-1 inhibitor reduced airway inflammation, tissue remodeling and airway hyperreactivity.[16,17]

The rs2227631 SNP itself had significant influence on the FEV₁/FVC ratio within our asthmatic subjects, which is contrast to the findings that this SNP alone was not associated with asthma risk. We did not have sufficient lung function data for non-asthmatic subjects to determine whether there is an effect in normal subjects who would presumably have less airway

inflammation and less remodeling potential. Furthermore, early childhood lower respiratory tract infection and the SNP had a joint effect on FEV₁ and the FEV₁/FVC ratio. These findings raise the question of whether the *rs2227631* SNP, in the context of viral insult, may cause increased production of airway *PAI-1* enough to affect lung development in infants and cause structural airway changes that lead to lower lung function in subjects with asthma.

This study has a number of limitations. While there was a dose effect for A alleles in our preliminary analyses, our numbers were not sufficiently large to analyze the AA-infection group separately for RSV bronchiolitis. AG and AA groups were combined for the primary analysis to provide more precise and stable estimates. Secondly, both the *PAI-1* SNP *rs2227631* [18,25] and the 4G/5G polymorphism are promoter site polymorphisms [19,20,26] in strong linkage disequilibrium ($D' = 0.97$), [18] making it difficult to determine which is functional even if the 4G/5G variant had been sequenced. However, others have used the *rs2227631* genotype as a proxy for the 4G/5G locus, and found it to be the variant which was most highly associated with *PAI-1* levels on GWAS analysis, suggesting that this may be the more important locus. [27] Third, the exposures of RSV/ bronchiolitis and LRI were based on a self-report questionnaire designed to elucidate infections resulting in lower respiratory symptoms in a child under the age of 2 years. This is based on the fact that most childhood respiratory illness is indeed due to viral pathogens as has been shown by the Hartert group. [24] While, it is also possible that a recall bias would have resulted in only more severe illnesses were reported, this is in keeping with our focus. Severe enough illness to require a visit to the physician increase the relevance of inflammation and *PAI-1*. Even if some subjects with less severe illness were systematically included in the “no severe infection group”, this would bias our analysis towards the null hypothesis, making our findings even more robust. Finally, it is also possible that asthmatic subjects may have greater recall of early wheezing illnesses which may increase the magnitude of the wheezing illness–asthma association. However, this effect should not bias the effect of genotype on asthma when studied within these symptomatic subjects as was evaluated in the subgroup analysis. This effect was clearly present, in contrast to the main analysis, which showed no genotype effect. Furthermore, other viruses may have caused symptomatic bronchiolitis and termed “RSV” by health care providers. [24] Over 70% of bronchiolitis is associated with RSV, [24] with most severe bronchiolitis associated with RSV or RSV/rhinovirus co-infection. [28] Thus, RSV is likely to be further enriched in this group beyond 70%. Regardless, our analyses were also consistent for LRI, a proof of the general principle. The importance of any early viral illness in the development of asthma in a susceptible host is underscored by a recent report that all viruses resulting in symptomatic illnesses in the first year of life (not just RV or RSV), increased the risk of asthma by age 7. [29]

Conclusion

In conclusion, a genetic variant of *PAI-1* which increases *PAI-1* production, together with either early life lower respiratory infection, was associated with asthma diagnosis, asthma exacerbations, and asthma severity based on reduced FEV₁/FVC ratio in our Latino population. The asthma associations for genotype-LRI were replicated in a smaller African American population. Further prospective studies are needed to replicate our RSV-genotype findings in other non-latino populations, and determine if *PAI-1* variants may serve as a biomarker of risk, which may provide impetus for clinical trials of primary prevention of asthma. In the interim, *PAI-1* genotype in combination with significant LRI, identifies individuals at increased risk of developing asthma. Studies are needed to determine whether interventions affecting airway responses at time of early life LRI in these at risk individuals will decrease the chances of developing asthma.

Supporting Information

S1 File. GALAI supporting data. This file includes data used to perform the primary analyses for this paper.
(TXT)

Acknowledgments

The authors acknowledge the families and patients for their participation and thank the numerous health care providers and community clinics for their support and participation in GALA II. In particular, the authors thank study coordinator Sandra Salazar; the recruiters who obtained the data: Duanny Alva, MD, Gaby Ayala-Rodriguez, Lisa Caine, Elizabeth Castellanos, Jaime Colon, Denise DeJesus, Blanca Lopez, Brenda Lopez, MD, Louis Martos, Vivian Medina, Juana Olivo, Mario Peralta, Esther Pomares, MD, Jihan Quraishi, Johanna Rodriguez, Shahdad Saeedi, Dean Soto, Emmanuel Viera, Ana Taveras; and Celeste Eng, who processed the samples and manages the data.

Author Contributions

Conceived and designed the experiments: SC JYM DYK SSO DH SS SH CE LKW MS WRC JR DS ST LNB EGB PA RK HJF.

Performed the experiments: SC JYM DYK SSO DH SS SH CE LKW MS WRC JR DS ST LNB EGB PA RK HJF.

Analyzed the data: JYM SC RK.

Contributed reagents/materials/analysis tools: SS WD.

Wrote the paper: SC JYM DYK SSO DYK DRT MS DH SS SH CE LKW MS WRC JR DS ST LNB EGB PA RK HJF MPY WD.

References

1. CDC (2011). Vital Signs.
2. Fagnani C, Annesi-Maesano I, Brescianini S, D'Ippolito C, Medda E, et al. (2008) Heritability and shared genetic effects of asthma and hay fever: an Italian study of young twins. *Twin Res Hum Genet* 11: 121–131. doi: [10.1375/twin.11.2.121](https://doi.org/10.1375/twin.11.2.121) PMID: [18361712](https://pubmed.ncbi.nlm.nih.gov/18361712/)
3. Thomsen SF, Kyvik KO, Backer V (2008) Etiological relationships in atopy: a review of twin studies. *Twin Res Hum Genet* 11: 112–120. doi: [10.1375/twin.11.2.112](https://doi.org/10.1375/twin.11.2.112) PMID: [18361711](https://pubmed.ncbi.nlm.nih.gov/18361711/)
4. Willemsen G, van Beijsterveldt TC, van Baal CG, Postma D, Boomsma DI (2008) Heritability of self-reported asthma and allergy: a study in adult Dutch twins, siblings and parents. *Twin Res Hum Genet* 11: 132–142. doi: [10.1375/twin.11.2.132](https://doi.org/10.1375/twin.11.2.132) PMID: [18361713](https://pubmed.ncbi.nlm.nih.gov/18361713/)
5. Hoffjan S, Nicolae D, Ober C (2003) Association studies for asthma and atopic diseases: a comprehensive review of the literature. *Respir Res* 4: 14. PMID: [14748924](https://pubmed.ncbi.nlm.nih.gov/14748924/)
6. Lockett GA, Holloway JW (2013) Genome-wide association studies in asthma; perhaps, the end of the beginning. *Curr Opin Allergy Clin Immunol* 13: 463–469. doi: [10.1097/ACI.0b013e328364ea5f](https://doi.org/10.1097/ACI.0b013e328364ea5f) PMID: [23945178](https://pubmed.ncbi.nlm.nih.gov/23945178/)
7. Wjst M, Sargurupremraj M, Arnold M (2013) Genome-wide association studies in asthma: what they really told us about pathogenesis. *Curr Opin Allergy Clin Immunol* 13: 112–118. doi: [10.1097/ACI.0b013e32835c1674](https://doi.org/10.1097/ACI.0b013e32835c1674) PMID: [23222155](https://pubmed.ncbi.nlm.nih.gov/23222155/)
8. Ober C, Vercelli D (2011) Gene-environment interactions in human disease: nuisance or opportunity? *Trends Genet* 27: 107–115. doi: [10.1016/j.tig.2010.12.004](https://doi.org/10.1016/j.tig.2010.12.004) PMID: [21216485](https://pubmed.ncbi.nlm.nih.gov/21216485/)
9. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, et al. (2008) Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 178: 667–672. doi: [10.1164/rccm.200802-309OC](https://doi.org/10.1164/rccm.200802-309OC) PMID: [18565953](https://pubmed.ncbi.nlm.nih.gov/18565953/)

10. Feldman AS, He Y, Moore ML, Hershenson MB, Hartert TV (2014) Toward Primary Prevention of Asthma: Reviewing the Evidence for Early-Life Respiratory Viral Infections as Modifiable Risk Factors to Prevent Childhood Asthma. *Am J Respir Crit Care Med*.
11. Caliskan M, Bochkov YA, Kreiner-Moller E, Bonnelykke K, Stein MM, et al. (2013) Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 368: 1398–1407. doi: [10.1056/NEJMoa1211592](https://doi.org/10.1056/NEJMoa1211592) PMID: [23534543](https://pubmed.ncbi.nlm.nih.gov/23534543/)
12. Cho SH, Hong SJ, Chen H, Habib A, Cho D, et al. (2014) Plasminogen activator inhibitor-1 in sputum and nasal lavage fluids increases in asthmatic patients during common colds. *J Allergy Clin Immunol* 133: 1465–1467, 1467 e1461-1462. doi: [10.1016/j.jaci.2013.11.009](https://doi.org/10.1016/j.jaci.2013.11.009) PMID: [24373352](https://pubmed.ncbi.nlm.nih.gov/24373352/)
13. Lee Chung H, Kim SY, Kim SG (2007) Vascular endothelial growth factor and plasminogen activator inhibitor-1 in children with recurrent early wheeze. *J Allergy Clin Immunol* 119: 1541–1542. PMID: [17445877](https://pubmed.ncbi.nlm.nih.gov/17445877/)
14. Eitzman DT, McCoy RD, Zheng X, Fay WP, Shen T, et al. (1996) Bleomycin-induced pulmonary fibrosis in transgenic mice that either lack or overexpress the murine plasminogen activator inhibitor-1 gene. *J Clin Invest* 97: 232–237. PMID: [8550840](https://pubmed.ncbi.nlm.nih.gov/8550840/)
15. Hattori N, Degen JL, Sisson TH, Liu H, Moore BB, et al. (2000) Bleomycin-induced pulmonary fibrosis in fibrinogen-null mice. *J Clin Invest* 106: 1341–1350. PMID: [11104787](https://pubmed.ncbi.nlm.nih.gov/11104787/)
16. Lee SH, Eren M, Vaughan DE, Schleimer RP, Cho SH (2012) A plasminogen activator inhibitor-1 inhibitor reduces airway remodeling in a murine model of chronic asthma. *Am J Respir Cell Mol Biol* 46: 842–846. doi: [10.1165/rcmb.2011-0369OC](https://doi.org/10.1165/rcmb.2011-0369OC) PMID: [22323366](https://pubmed.ncbi.nlm.nih.gov/22323366/)
17. Miyamoto S, Hattori N, Senoo T, Onari Y, Iwamoto H, et al. (2011) Intra-airway administration of small interfering RNA targeting plasminogen activator inhibitor-1 attenuates allergic asthma in mice. *Am J Physiol Lung Cell Mol Physiol* 301: L908–916. doi: [10.1152/ajplung.00115.2011](https://doi.org/10.1152/ajplung.00115.2011) PMID: [21926267](https://pubmed.ncbi.nlm.nih.gov/21926267/)
18. Kathiresan S, Gabriel SB, Yang Q, Lochner AL, Larson MG, et al. (2005) Comprehensive survey of common genetic variation at the plasminogen activator inhibitor-1 locus and relations to circulating plasminogen activator inhibitor-1 levels. *Circulation* 112: 1728–1735. PMID: [16172282](https://pubmed.ncbi.nlm.nih.gov/16172282/)
19. Cho SH, Hall IP, Wheatley A, Dewar J, Abraha D, et al. (2001) Possible role of the 4G/5G polymorphism of the plasminogen activator inhibitor 1 gene in the development of asthma. *J Allergy Clin Immunol* 108: 212–214. PMID: [11496236](https://pubmed.ncbi.nlm.nih.gov/11496236/)
20. Pampuch A, Kowal K, Bodzenta-Lukaszyk A, Di Castelnuovo A, Chyczewski L, et al. (2006) The -675 4G/5G plasminogen activator inhibitor-1 promoter polymorphism in house dust mite-sensitive allergic asthma patients. *Allergy* 61: 234–238. PMID: [16409202](https://pubmed.ncbi.nlm.nih.gov/16409202/)
21. Drake KA, Torgerson DG, Gignoux CR, Galanter JM, Roth LA, et al. (2014) A genome-wide association study of bronchodilator response in Latinos implicates rare variants. *J Allergy Clin Immunol* 133: 370–378. doi: [10.1016/j.jaci.2013.06.043](https://doi.org/10.1016/j.jaci.2013.06.043) PMID: [23992748](https://pubmed.ncbi.nlm.nih.gov/23992748/)
22. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, et al. (2013) Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. *Am J Respir Crit Care Med* 188: 309–318. doi: [10.1164/rccm.201302-0264OC](https://doi.org/10.1164/rccm.201302-0264OC) PMID: [23750510](https://pubmed.ncbi.nlm.nih.gov/23750510/)
23. Staat MA (2002) Respiratory syncytial virus infections in children. *Semin Respir Infect* 17: 15–20. PMID: [11891515](https://pubmed.ncbi.nlm.nih.gov/11891515/)
24. Miller EK, Gebretsadik T, Carroll KN, Dupont WD, Mohamed YA, et al. (2013) Viral etiologies of infant bronchiolitis, croup and upper respiratory illness during 4 consecutive years. *Pediatr Infect Dis J* 32: 950–955. doi: [10.1097/INF.0b013e31829b7e43](https://doi.org/10.1097/INF.0b013e31829b7e43) PMID: [23694832](https://pubmed.ncbi.nlm.nih.gov/23694832/)
25. Su S, Chen S, Zhao J, Huang J, Wang X, et al. (2006) Plasminogen activator inhibitor-1 gene: selection of tagging single nucleotide polymorphisms and association with coronary heart disease. *Arterioscler Thromb Vasc Biol* 26: 948–954. PMID: [16424345](https://pubmed.ncbi.nlm.nih.gov/16424345/)
26. Nie W, Li B, Xiu QY (2012) The -675 4G/5G polymorphism in plasminogen activator inhibitor-1 gene is associated with risk of asthma: a meta-analysis. *PLoS One* 7: e34385. doi: [10.1371/journal.pone.0034385](https://doi.org/10.1371/journal.pone.0034385) PMID: [22479620](https://pubmed.ncbi.nlm.nih.gov/22479620/)
27. Huang J, Sabater-Lleal M, Asselbergs FW, Tregouet D, Shin SY, et al. (2012) Genome-wide association study for circulating levels of PAI-1 provides novel insights into its regulation. *Blood* 120: 4873–4881. doi: [10.1182/blood-2012-06-436188](https://doi.org/10.1182/blood-2012-06-436188) PMID: [22990020](https://pubmed.ncbi.nlm.nih.gov/22990020/)
28. Hasegawa K, Mansbach JM, Teach SJ, Fisher ES, Hershey D, et al. (2014) Multicenter study of viral etiology and relapse in hospitalized children with bronchiolitis. *Pediatr Infect Dis J* 33: 809–813. doi: [10.1097/INF.000000000000293](https://doi.org/10.1097/INF.000000000000293) PMID: [24577039](https://pubmed.ncbi.nlm.nih.gov/24577039/)
29. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H (2015) Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol* 136: 81–86.e84. doi: [10.1016/j.jaci.2015.02.024](https://doi.org/10.1016/j.jaci.2015.02.024) PMID: [25910716](https://pubmed.ncbi.nlm.nih.gov/25910716/)