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TITLE: **Pathways to Disease: The Biological Consequences of Social Adversity on Asthma in Minority Youth**

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14. ABSTRACT Asthma incidence is increasing worldwide and disproportionately affects disadvantaged and minority populations. There is overrepresentation in the Active Duty military of low income and minority populations, including African Americans and Latinos. These populations experience the greatest social adversities and have significant asthma burden. The etiology of asthma-related disparities is multifactorial and known to be affected by poverty and its associated exposures. Chronic exposure to social adversities may trigger a stress response resulting in modulation of immune and hormonal responses and disruption of the body's microbiome. This toxic stress response is likely to be unique in each racial/ethnic group and depend on genetic susceptibility, the environment, and personal upbringing. The current proposal will address the cause, treatment, and prevention of asthma in high-risk populations. Aim 1 will focus on the immune system and hypothalamus-pituitary-adrenal axis response to social adversities and the effect on asthma outcomes (n=1000). Aim 2 will focus on the effect of social adversities on the microbiome and if the differences observed are associated with asthma (n=200). The proposal will allow for us to delineate the pathways by which social adversities impart their effects and identify points for intervention to improve asthma related outcomes.					
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1. Introduction

Asthma incidence is increasing worldwide and disproportionately affects disadvantaged and minority populations. The etiology of asthma-related disparities is multifactorial and known to be affected by poverty and its associated exposures. There is overrepresentation in the Active Duty military of low income and minority populations, including African Americans and Latinos. These populations experience the greatest social adversities and have significant asthma burden, including higher asthma mortality. Chronic exposure to social adversities may trigger a toxic stress response resulting in modulation of the immune and hormonal response and disruption of the body's microbiome, both of which have been shown to negatively affect disease outcomes. This toxic stress response is likely to be unique in each racial/ethnic group and depend on genetic susceptibility, the environment, and personal upbringing. The current proposal will address the cause, treatment, and prevention of asthma in high-risk populations. This will be achieved by delineating the pathways by which social adversities impart their effects on asthma susceptibility and morbidity in minority populations. Aim 1 will focus on the immune system and hypothalamus-pituitary-adrenal axis response to social adversities and their effect on asthma susceptibility and morbidity. We have measured 526 of the proposed total of 1000 samples. Aim 2 will focus on the effect of social adversities on the microbiome and if the differences observed are associated with asthma. The measurement of the microbiome (n=200) will start in November 2016. The proposal will allow for better identification of high-risk populations and development of interventions that target the modifiable aspects of social adversities to effectively improve asthma outcomes.

2. Keywords

Asthma, Adolescents, Young Adults, Chronic Stress, Socioeconomic Stress, Toxic Stress, Minority Health, Health Disparities, Protein-based Biomarkers, Microbiome, Allostatic Load.

3. Accomplishments

○ What were the major goals of the project?

There are two major goals for the study that align with the Specific Aims. The **first major goal** is to measure biomarkers related to the immune and neuroendocrine system. To date, we have immune- and neuroendocrine-related biomarkers on 526/1000 participants. We anticipate the measurements will be complete by January 31, 2017.

The **second major goal** is to measure and examine the oral microbiome in relation to measures of psychosocial and socioeconomic stress and asthma. We will start amplification of the V4 16S rRNA hypervariable region in November 2016. This will be completed by March 2017.

○ What was accomplished under these goals?

There are two major goals for the study that align with the Specific Aims. The **first major goal** is to measure biomarkers related to the immune and neuroendocrine system. The biomarkers were measured in our laboratory using immunoassays or sent to our clinical laboratory. We have measure immune- and neuroendocrine-related biomarkers on 526/1000 participants and anticipate completion by January 31, 2017.

As part of a preliminary study for this proposal, we have measured TNF-alpha, a pro-inflammatory cytokine associated with both asthma and psychosocial stress, in our African American participants with asthma (n=576). As part of this proposal, we have completed an analysis examining the effect of perceived racial discrimination on bronchodilator response (a measure of airway contractility) to albuterol (the mainstay rescue drug for asthma) among African American youth with asthma. We know that asthma is a multifactorial disease with varying risk profiles and outcomes, and thus, phenotypes. However, it is unknown which of these asthma phenotypes are vulnerable to psychosocial stress, the main exposure of interest for this proposal and a well described independent contributor to asthma morbidity. Almost half of participants (48.8%) reported experiencing racial discrimination. Those reporting discrimination were older (median age 15.4 versus 12.1 years, $p<0.001$), had a history of *in utero* smoke exposure (22.1 versus 15.3%, $p=0.036$), and had poorly controlled asthma (50.2 versus 33.9%; $p<0.001$). In the adjusted analysis, the mean BDR difference between participants reporting discrimination and those who did not was 1.70% (95%CI: 0.36-3.03%). However, this difference varied with TNF- α status ($p=0.040$). Among individuals with TNF- α high asthma, we observed a 2.78% greater mean BDR among those reporting perceived discrimination than those not reporting discrimination (95%CI: 0.79-4.77%). This difference was not seen in the TNF- α low asthma group (0.66%, 95%CI: -1.19-2.51%; **Table**). This is clinically important, as those who are at risk of poor asthma outcomes and were previously thought to be unresponsive to asthma medications may actually be responsive and may benefit from adjunct behavioral or environmental interventions. These results support screening for psychosocial stress in those with moderate-severe asthma as it may reclassify asthma type and delineate a treatment path. These results were accepted as an oral abstract at the 2016 UCSF Health Disparities Forum (San Francisco, CA) and were recently submitted for review to a scientific journal in the form of a manuscript.

Table: Mean Difference in Bronchodilator Response[^] and 95% CI for Reports of Racial Discrimination and according to TNF- α status for SAGE II Participants with Asthma (2006-2014)

		TNF- α Status ²		
		Adjusted ¹	Low ¹	High ¹
Racial Discrimination	Never	Reference	Reference	Reference
	Any	1.70 (0.36, 3.03)	0.78 (-1.07, 2.63)	2.78 (0.79, 4.77)

[^] Bronchodilator response: mean percentage change in measured FEV₁ before and after albuterol administration, using the post-albuterol spirometry with the maximal change.

¹ adjusted for sex, age, maternal education, recruitment center, *in utero* smoke exposure, daycare attendance, baseline lung function, controller medication use, African ancestry, TNF- α mean, and biomarker storage time.

² p-interaction = 0.04

The **second major goal** is to measure and examine the oral microbiome in relation to measures of psychosocial and socioeconomic stress and asthma. We will deep sequence the 16S rRNA gene from the DNA in saliva samples of participants. We have already extracted the DNA on all samples and have obtained a mock bacteria community for comparison and quality control. We will start amplification of the V4 16S rRNA hypervariable region in November 2016. This will be completed by March 2017. Samples will be sequenced by pair-end 300 base pair reads in a

MiSeq sequencer. Once measured, we will define the oral microbiome in terms of richness, diversity and bacterial taxonomy as it relates to stress exposures and asthma.

- **What opportunities for training and professional development has the project provided?** *Nothing to Report*
- **How were the results disseminated to communities of interest?**

The participants included in this study are from clinics that serve predominantly under-insured minority communities that experience an excess of social adversities and chronic stress. The results of the preliminary study examining the role of perceived discrimination on drug response among African American youth with low and high TNF-alpha were shared with clinical providers and outreach coordinators from the Center of Youth Wellness (Bayview neighborhood, San Francisco, CA) and UCSF Children's Hospital Oakland (Oakland, CA) in the format of a journal club. We were able to discuss the potential clinical implications and the necessary next steps to confirm our findings.

- **What do you plan to do during the next reporting period to accomplish the goals?**

For **project goal 1** we will complete measurement of the inflammatory and neuroendocrine biomarkers by January 31, 2017. We will then 1) examine how the biomarkers differ by asthma diagnosis (case/control study), and 2) determine if these biomarkers differ by stress exposure in those with and without asthma (stratified analysis). These analyses will take place in the last 4 months of the current reporting period.

For **project goal 2** we will start measurement of the oral microbiome using sequencing of the 16S rRNA region in November 2016 and anticipate completion by March 2017. Quality control and identification of the diversity, richness, and abundance of the microbiome data will be completed in the last 4 months of the current reporting period.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

The preliminary results, the content expertise, and the infrastructure developed from this proposal led to the successful funding for a longitudinal study of early-life exposure to adversity and health, including asthma. The TARA Health Foundation awarded \$4.8 Million dollars to establish the Bay Area Research Consortium on Toxic Stress and Health; UCSF (Thakur) received \$819,415 to examine biomarkers as they relate to social adversity, stress, and health (study period: 2015-2019). This study will comprehensively measure exposure to trauma and adversity in childhood that are commonly associated with post-traumatic stress disorder in adulthood and will follow the enrolled children longitudinally. We will obtain biospecimens at several time points over the course of the study and measure inflammatory and neuro-endocrine biomarkers, the microbiome, and telomere length and relate these biomarkers to the measured exposures to adversity and stress. The selection of and methods to measure the biomarkers were directly informed by this study and represent the natural next step from the current study. Together, these two studies have the opportunity to change the way we think about social

adversities and health by providing a biological framework and identifying critical points for intervention.

- **What was the impact on other disciplines?** *Nothing to Report*
- **What was the impact on technology transfer?** *Nothing to Report.*
- **What was the impact on society beyond science and technology?**

The results of this study have the potential to have great impact on how we classify asthma and determine treatment path. The results from our TNF-alpha and discrimination study provides support for screening for psychosocial stress in those with moderate to severe asthma as those who are at risk of poor asthma outcomes and were previously thought to be unresponsive to asthma medications may actually be responsive and may benefit from adjunct behavioral or environmental interventions.

5. Changes/Problems

- **Changes in approach and reasons for change:** *Nothing to Report.*
- **Actual or anticipated problems or delays and actions or plans to resolve them**

The proposed timeline for our project was delayed. We experienced an initial delay of 3 months while the Department of Defense's Human Research Protection Office completed their review of the project. This review resulted in a local (UCSF) Institutional Review Board Amendment of the project and we were granted approval from the HRPO at the end of December 2015. After selecting a subset of our study population for evaluation, we experienced a second delay of almost three months in setting up our account for Clinical lab testing. Since we were able to prepare the selected samples for biomarker testing in the interim, we are on track to complete our biomarker by January 31, 2017.

- **Changes that had a significant impact on expenditures**

While there have not been any significant changes in the overall cost of the project, the delays listed above have shifted the overall timeline of expenditures by six months.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:** *Nothing to Report*
- **Significant changes in use or care of human subjects:** *Nothing to Report*
- **Significant changes in use or care of vertebrate animals** N/A
- **Significant changes in use of biohazards and/or select agents** N/A

6. Products:

- **Publications, conference papers, and presentations:** *Nothing to Report*
- **Journal publications.**

S Carlson, LN Borrel, SS Oh, C Eng, A Davis, K Meade, HJ Farber, E Brigino-Buenaventura, S Thyne, S Sen, MA LeNoir, N Burke-Harris, EG Burchard, N Thakur. Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma. September 2016. *Under Review.*

- **Books or other non-periodical, one-time publications:** *Nothing to Report*
- **Other publications, conference papers, and presentations.**

S Carlson, LN Borrel, SS Oh, C Eng, A Davis, K Meade, HJ Farber, E Brigino-Buenaventura, S Thyne, S Sen, MA LeNoir, N Burke-Harris, EG Burchard, N Thakur. Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma. UCSF Health Disparities Research Symposium 2016. San Francisco, CA October 2016.

- **Website(s) or other Internet site(s):** *Nothing to Report*
- **Technologies or techniques:** *Nothing to Report*
- **Inventions, patent applications, and/or licenses:** *Nothing to Report*
- **Other Products**

Database: With this study we have added plasma-based biomarker measurements to the SAGE II and GALA II datasets. Once the microbiome data is measured this will also be added to the datasets. These two pieces of the information will allow it to be possible to perform analyses across multiple levels of data ranging from the plasma-based biomarkers to environmental data.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	Neeta Thakur
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-6126-6601
Nearest person month worked:	3
Contribution to Project:	Dr. Thakur oversaw the measurement of biomarkers, came up with the research question and analytical plans for the preliminary study.
Funding Support:	NHLBI K23 Career Development Award, Parker B. Francis Fellowship Program
Name:	Sam Oh
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	0000-0002-2815-6037
Nearest person month worked:	1
Contribution to Project:	Dr. Oh

Funding Support:	NIH/ NIMHD; NIH/NHLBI; NIH/NIEHS; DOD; Harvard Pilgrim Health Care, Inc.
Name:	Celeste Eng
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Ms. Eng
Funding Support:	INO Therapeutics; NIH/NIMHD; NIH/NHLBI: University of California Tobacco Related Disease Program, Tara Foundation *
Name:	DongLei Hu
Project Role:	Biostatistician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Hu
Funding Support:	INO Therapeutics; NIH/NHLBI; NIH/NCI; DOD; City of Hope/NIH/NCI; NIH/NIMHD; Harvard Pilgrim Health Care, Inc.
Name:	Sonia Carlson
Project Role:	Medical Student
Researcher Identifier (e.g. ORCID ID):	n/a
Nearest person month worked:	4
Contribution to Project:	Ms. Carlson
Funding Support:	NIH/NIMHD UCSF PROF-PATH

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

The DOD effort of 2.4 CM was previously subsumed by the NHLBI K12 awarded to UCSF. Dr. Thakur has now been awarded her own Career Development Award (K23) from the NHLBI which has replaced the support previously received by the UCSF K12 program. See Other Support below:

ACTIVE

Recognition Award for	11/01/2014 – 11/29/2016	2CM
Outstanding Early Career Investigators	\$40,000/total	effort subsumed by K23
American Thoracic Society		

Allostatic Load and Asthma: Chronic Stress and Asthma in Minority Children

Project Goals: The goal of the Recognition Award is to fund researchers who had a Career Development Award that was near fundable level but not awarded from the National Institute of Health or an equivalent organization. This support will be used for ancillary supplies to measure cytokines in African American study participants to better elucidate the inflammatory response to social stressors.

Role: Principal Investigator

Parker B. Francis Fellowship Program	7/1/2015-6/30/2018	4CM
Francis Family Foundation	\$156,000/total	effort subsumed by K23

Social Adversity and Asthma: A new phenotype?

Goals: The goal1 of this award is to 2) identify risk factors for poor asthma outcomes in African American and Latino children that are related to social and environmental exposures, 2) test a limited set of inflammatory biomarkers to determine if they are elevated in the presence of specific stress exposures, and 3) Determine if there is an asthma phenotype susceptible to social and environmental stress.

Role: Principal Investigator/ Career Development Award Recipient

PR141896 Discovery Award (Thakur)	09/31/2015-03/31/2017	2.4CM
Department of Defense	\$200,000 direct/year	effort subsumed by K23
		NO SALARY SUPPORT

Pathways to Disease: The Biological Consequences of Social Adversity on Asthma in Minority Youth

Project Goals: The goal of this award is to better delineate the biological pathways of stress related to socioeconomic and environmental stress among urban, minority of youth with asthma. This study will examine 1) the immune and neuro-endocrine response and 2) the microbiome in response to chronic exposure to psychosocial stress.

K23HL125551-01A1 (Thakur)	07/01/2016 – 06/30/2021	9 CM
NIH/NHLBI	\$165,000 direct/year	

Social Adversities and Asthma: A New Phenotype?

Project Goals: The goals of this project are to 1) identify individual- and community-level risk factors for asthma among disadvantage, minority youth; 2) define a profile of characteristics, which includes biomarker data, that will better identify individuals at high risk for poor asthma outcomes who are from communities burdened by social adversities; and 3) examine the asthma-related outcomes in individuals with the identified phenotype.

Tara Health/Center for Youth Wellness (Thakur)	9/1/2015-2/28/2019	.6CM
	\$755,141/total	

Title: Adverse Childhood Experiences (ACEs) BioCore Bank

Goals: The lack of standardized, high-quality biospecimens is widely recognized as a significant roadblock in biomedical research. We propose a full-service processing and banking laboratory, the ACEs BioCore Bank (ABC Bank), to facilitate the advancement of the study of ACEs . The ABC Bank will be a high-functioning, multidisciplinary operation with the overarching purpose

to achieve the following goal: Carefully collect, process, test and store high-quality biospecimens across multiple sites in a consistent manner.

Role: Principal Investigator

OVERLAP: Her Concurrent Effort from the ATS, Parker Francis Fellowship, DOD awards are included in the K23 effort. The project goals are to identify social and environmental risk factors for asthma, better delineate pathways by which social adversities impact asthma, and, using a profile of demographic, biologic, and clinical data, identify individuals at risk for poor asthma outcomes. The goals of these awards are directly in line with the overall goal of the K23.

- **What other organizations were involved as partners?**

Organization Name: Center for Youth Wellness

Location of Organization: *San Francisco, CA*

Partner's contribution to the project

In-kind support: SAGEII and GALAII include a total of 6,500 participants. This proposal allows for the measurement of biomarkers in 1000 of these participants. The CYW provided assays and associated materials for the measurement of biomarkers in an additional 750 individuals from the SAGEII study.

8. Special Reporting Requirements: NA

9. Appendix:

See attached submitted manuscript "Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma."

Appendix

Cover Letter

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Other related papers from study population:

Title page

Title: Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma.

Authors:

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Manuscript word count: 2666

Conflict of Interest: The authors have no conflicts of interest to disclose.

Abstract

Importance: Asthma is a multifactorial disease composed of several endotypes, pathways to disease, with varying risk profiles and outcomes. African Americans experience a high burden of asthma and of psychosocial stress, including racial discrimination. It is unknown which endotypes of asthma are vulnerable to psychosocial stress.

Objective: We aim to examine the association between self-report of racial discrimination and bronchodilator response (BDR) among African American youth with asthma and whether this association varies with tumor necrosis factor alpha (TNF- α) level.

Design: A case-only analysis of youth with asthma from the Study of African Americans, Asthma, Genes and Environments (SAGE) recruited between 2008 and 2014.

Setting: San Francisco Bay Area multicenter study.

Participants: African American youth ages 8 to 21 years (n=576).

Main outcome and Measures: Perceived racial discrimination was assessed by the Experiences of Discrimination questionnaire. Maximal BDR was specified as the mean percentage change in forced expiratory volume in one second before and after albuterol administration using spirometry. TNF- α was measured on stored biospecimens and specified as high/low levels based on the study population mean. Multivariable linear regression was used to examine the association between discrimination and BDR adjusted for selected characteristics and risk factors. An interaction term between TNF- α levels and discrimination was tested in the final model.

Results: Almost half of participants (48.8%) reported experiencing racial discrimination. In adjusted analysis, participants reporting racial discrimination had a 1.7 (95% CI: 0.36-3.03) higher BDR mean than those not reporting discrimination. However, we found heterogeneity of this association according to TNF- α levels (p-interaction=0.040): Among individuals with TNF- α high level, we observed a 2.78 higher BDR mean among those reporting perceived discrimination compared with those not reporting discrimination (95%CI: 0.79-4.77). This association was not observed among those with TNF- α low level.

Conclusion and Relevance: We found BDR to be increased in participants reporting discrimination and this association was limited to African American youth with TNF- α high asthma, an endotype thought to be resistant to traditional asthma medications. These results support screening for psychosocial stress in those with asthma as it may reclassify disease pathogenesis.

Text

INTRODUCTION:

African American children have one of the highest asthma prevalence and mortality rates in the U.S.¹ African Americans experience higher prevalence of asthma (11.2%) than Non-Hispanic whites (7.7%). This is also true for mortality (0.23 per 1000 individuals with asthma in African Americans versus 0.13 per 1000 individuals with asthma in whites).¹ While there are well known risk factors for these disparities, psychosocial stress seems to be surfacing as an independent risk factor. African Americans experience an excess of psychosocial stress compared to non-Hispanic whites.² Psychosocial stress negatively impacts youth and is associated with wheezing and increased exacerbations among youth with asthma.³ However, this response to psychosocial stress is inconsistent.^{4,5} This inconsistency may result from the heterogeneous nature of asthma. Asthma is no longer thought of as a single disease, but as a disorder composed of distinct asthma types with varying pathophysiology. These varying pathways are endotypes of asthma and are thought to reflect a particular biologic mechanism and are linked to specific health outcomes such as inhaled corticosteroid response and frequent exacerbations.⁶ Psychosocial stress may affect asthma outcomes differently according to these asthma endotypes.

A commonly used outcome of asthma is bronchodilator response (BDR), which aids in diagnosis,⁷ in assessing responsiveness to inhaled corticosteroids, and as a predictor of future lung function.⁸ Previous research has shown that a $BDR \geq 10\%$ is associated with poor asthma control.⁹ Therefore, BDR is thought to be useful as a clinical tool to identify individuals at risk of poor asthma outcomes. Youth who experience psychosocial stress secondary to social

disadvantage tend to have poor asthma control.¹⁰ Thus, it is important to measure and assess the effects of psychosocial stress on BDR.

Racial discrimination is a form of psychosocial stress that may put certain youth at higher risk for poor asthma outcomes.¹¹ Experiences of racial discrimination are biased treatment associated with individual characteristics such as the color of skin.¹² A high proportion of minority youth (up to 88%) have reported racial discrimination.¹³ We have previously demonstrated that racial discrimination is independently associated with increased odds of asthma in African Americans.¹¹ Others have reported that experiences of discrimination, whether directed toward the individual or to family members, were associated with worse asthma symptoms and control.¹⁴

For this study, we focused on a moderate-to-severe asthma endotype that is neutrophilic and is associated with up-regulation of Tumor Necrosis Factor Alpha (TNF- α).¹⁵ This endotype is characterized as having lower lung function.¹⁶ It has been previously described that even within a moderate-to-severe asthma group, a subgroup characterized by elevated TNF- α had higher reports of symptoms and excessive health care use compared to those with lower TNF- α .¹⁷ Even within one endotype of asthma there are overlapping mechanisms,¹⁸ and thus, individuals may respond differently to the same trigger, including psychosocial stress.

OBJECTIVE: We aimed to examine the association of perceived discrimination with BDR to albuterol among youth and whether this association varies with TNF- α levels.

DESIGNS AND METHODS

Study Population

Participants for this study were enrolled through the Study of African Americans, Asthma, Genes & Environments (SAGE) between 2008 and 2014. This parent study is a case-control study designed to examine the complex genetic and socio-environmental contributors to asthma prevalence, control and severity among minority children and adolescents. The SAGE study recruited African American youth with and without asthma aged 8-21 years of age from urban regions in the San Francisco Bay Area. Asthma was defined as physician diagnosis and report of symptoms and medication use within the two years prior to recruitment.⁷ Participants must have self-identified all four grandparents as African American to be eligible for the study. Those in the third trimester of pregnancy, with ≥ 10 pack-year smoking history, and current smokers were not eligible (**eTable 1**). All local institutional review boards approved the study and all parents/participants provided appropriate written consent/assent.

Assessment of Perceived Discrimination

Trained interviewers administered comprehensive questionnaires to the parents/caretakers of the participants to collect socio-demographic information, medical histories, and environmental exposure-related information. The primary exposure for this analysis was perceived discrimination, ascertained using the Experiences of Discrimination (EOD) Questionnaire.¹⁹ For the purpose of this study, we included questions pertinent to a pediatric population, as follows: Have you ever experienced discrimination, been prevented from doing something, or been hassled or made to feel inferior, in any of the following situations because of your race, ethnicity, color, or language? (1) At School; (2) Getting medical care; (3) Getting services in a store or

restaurant; and (4) On the street or in a public setting; with choice for each question of Yes or No. Consistent with a previous study,¹¹ experiences of discrimination were defined as none or any (affirmative answer to at least one situation).

Assessment of biomarkers

Biomarkers were measured in stored frozen (-80°C) plasma specimens with storage times ranging from 3.1-9.5 years. Specimens were stored in multiple aliquots to minimize freeze-thaw cycle. TNF- α has been shown to remain stable over prolonged storage periods.²⁰ TNF- α was measured using a Magnetic Luminex Performance Assay from R&D systems in duplicate (n=29) or triplicate form (n=4). We excluded 16 individuals with failed assay (n=5) or >10% variation in duplicate/triplicate value (n=11). For individuals with $\leq$ 10% variation in measured values (n=8), we randomly selected one duplicate value to include as the measured value. Averages of remaining duplicate/triplicate values were used to determine the final measured TNF- α level for each individual. Storage time of TNF- α was added as a covariate and calculated based on date of recruitment and biomarker processing time. For this analysis and consistent with previous studies,^{21,22} we classified individuals as TNF- α high and low based on being above or below the study population mean of 1.42 pg/ml.

Covariates

Informed by previous studies,²³⁻²⁵ age, sex, *in utero* smoke exposure (i.e., maternal smoking during pregnancy), socioeconomic status, early life exposure to daycare, body mass index, and African ancestry were considered as potential confounders. We used maternal educational attainment as a stable measure of socioeconomic status²⁶ and it was categorized as less than high

school graduate, high school graduate, and some college or greater. Body mass index (BMI) was specified as BMI percentiles obtained using sex- and age- specific curves.²⁷ Estimates of African ancestry were obtained for each participant using an unsupervised analysis in ADMIXTURE assuming three ancestral populations. We used reference haplotypes from European and African individuals from HapMap phase II. For this analysis, we included a measurement of baseline lung function and the report of asthma controller medications. Baseline lung function was measured using spirometry per American Thoracic Society guidelines. We used an individual's percent of predicted FEV₁ measurement with a cutoff of 80%. The brief medication questionnaire²⁸ was used to ascertain reported controller medications use and we included this variable as a covariate when examining asthma control. Controller medication use was defined as the report of inhaled corticosteroid, leukotriene inhibitor, or long-acting-beta agonist in the two weeks prior to recruitment. Finally, recruitment site was also included as a covariate.

OUTCOME MEASURES:

Pulmonary Function Measures and Bronchodilator Response

The primary outcome for this study was maximal BDR to albuterol. All asthma medications were held for 12 h before spirometry. Per the American Thoracic Society recommendations, pulmonary function was measured before albuterol administration and then repeated 15 min after administration of four puffs of albuterol (90 µg per puff).²⁹ Spirometry was repeated a third time after a second dosage of albuterol (two puffs if < 16 years old or four puffs if ≥ 16 years old). We assessed the maximal BDR as the mean percentage change in measured FEV₁ before and after albuterol administration, using the post-albuterol spirometry with the maximal change. For analytical purposes, BDR was specified as continuous.

By 2014, there were 1009 eligible participants with asthma and stored biospecimens in SAGE II. Participants were excluded from the analysis if they were missing perceived discrimination questions (n=194), variables related to SES (n=20), environmental exposure data (daycare attendance and *in utero* smoke exposure; n=45), pulmonary function measures (n=69), or had inconclusive or missing TNF- α measurements (n=83), or other covariate information (n=22). These exclusions yielded an analytical sample size of 576. When comparing records for excluded and included participants, excluded participants were older (14.5 versus 13.5 years, $p = 0.008$), more likely to report *in utero* tobacco smoke exposure (24.1 versus 18.6%, $p = 0.034$), and were less likely to have mothers with higher education (50.7 versus 60.9%, $p < 0.001$).

Statistical Analysis

Descriptive statistics for cases according to reports of discrimination were calculated. Significance differences and associations were determined using Student t-test for continuous variables with a normal distribution, Kruskal-Wallis test for variables non-normally distributed, and chi square tests for categorical variables with normal distribution. Covariates significantly associated with BDR ($p < 0.2$) were included in the final model. We used linear regression to estimate the association between the report of perceived discrimination and BDR before and after controlling for selected covariates. To determine whether these associations vary with TNF- α level, an interaction term between discrimination and TNF- α was tested in the final model. Significance of main effects was determined at 0.05 and for interaction terms at 0.10. All analyses were conducted with R 3.1.2.³⁰

RESULTS:

Baseline Study Characteristics

Selected characteristics of participants without and with experiences of discrimination are displayed in **Table 1**. Approximately half (48.8%) of our participants reported perceiving racial discrimination in any setting at some point in their life. When compared with youth who do not experience discrimination, participants with experiences of discrimination were older (median age 15.4 versus 12.1 years, $p < 0.001$), more likely to be exposed to *in utero* smoke (22.1 versus 15.3 %, p -value = 0.036) and had mothers with lower levels of educational attainment compared to those who did not have experiences of discrimination (67.3 versus 54.9%, p -value = 0.008). Participants who experience discrimination were more likely to have very poorly controlled asthma (50.2 versus 33.9%; $p < 0.001$). Moreover, the mean BDR was higher among those reporting discrimination (10.8%, SD 9.8%) than among those not reporting perceived discrimination (8.9%, SD 7.8%; $p = 0.006$). There was no association between TNF- α and discrimination.

Bronchodilator Response and Perceived Discrimination

Participants who reported any discrimination had a 1.7% (95%CI 0.36 – 3.03) greater BDR compared with children not reporting discrimination after adjusting for sex, age, maternal education, recruitment center, *in utero* smoke exposure, daycare attendance, baseline lung function, controller medication use, African ancestry, TNF- α mean, and biomarker storage time (**Table 2**). We observed a significant heterogeneity of the association between perceived discrimination and BDR according to TNF- α status (High/Low; p -interaction = 0.040). For participants in the TNF- α high group, those reporting discrimination had a 2.78 (95%CI: 0.79-

4.77, $p=0.007$) greater mean BDR to albuterol than those not reporting discrimination. This association was not observed among those in the TNF- α low group (**eFigure 1**). Selected characteristics of participants with TNF- α high and low asthma are reported in the supplement and displayed in **eTable 2**.

DISCUSSION:

In this study, we observed an association between reports of discrimination and mean BDR. However, a significant increased mean BDR among those reporting discrimination was observed only among participants in the TNF- α high group. Our results corroborated previous studies suggesting that discrimination as a psychosocial stressor affects health in youth, including asthma outcomes.^{3,11} In contrast to a previous study which showed that psychosocial stress reduces BDR,³¹ we observed an increase in BDR in participants with TNF- α high asthma and reports of perceived discrimination. This supports the theory that asthma is heterogeneous and that this heterogeneity extends to the endotypes already identified, such as TNF- α asthma.¹⁸

Our study shows that screening for psychosocial stress may be important among those with moderate-severe asthma. This is clinically relevant as different treatments or interventions may be applied to this difficult to control group. There is evidence that adjunct socio-behavioral interventions to traditional asthma management improve outcomes;³² however, these interventions are perceived as time and labor intensive. By identifying a risk factor profile including measures of psychosocial stress and inflammatory biomarkers, we may be better equipped to identify individuals who are most susceptible to psychosocial stress, and thus, more

likely to benefit from such therapy. In addition, identification of such profile provides illumination on the various biological mechanisms to the development of TNF- α high asthma. The different responses to medication among individuals thought to represent one endotype of asthma generate speculation on mechanisms for the various asthma endotypes.^{33,34} One pathway may involve inflammatory and neuro-endocrine mechanisms that lead to different asthma endotypes.³⁵ These pathways may explain the variation in response to stress brought on by childhood upbringing, environment, genetics and race/ethnicity. Biomarkers of stress involved in systemic inflammation, such as TNF- α , have been shown to be elevated in acute asthma exacerbations in comparison to individuals with well controlled asthma.³⁶ Additionally, individuals perceiving psychosocial stress have been shown to have elevated levels of TNF- α compared to those not perceiving psychosocial stress.³⁷ Psychosocial stress secondary to perceived discrimination may enhance airway inflammation in asthma by modulating immune cell function through hormonal pathways.^{35,38} One study shows how social stress potentially alters lung function, increases levels of TNF- α , and decreases drug response in the treatment of asthma.³⁸ This has been traditionally thought of as TNF- α -high-asthma, which has been known to be severe and non-responsive to asthma medications.¹⁷

There are several limitations in the study. First, the cross-sectional design of our study limits our ability to identify causal relationships between our exposure and outcome. Second, because asthma is an inflammatory disease that is susceptible to acute and chronic changes in stress (physical and psychosocial),³⁹ TNF- α may be elevated in youth with asthma as a result of the underlying disease and altered by controller medications. Our analyses included controller medication use and a marker of lung function severity as covariates to help address these issues.

Third, because participants were selected based on disease status, it is unlikely that our exclusions have affected our results. Finally, the discrimination questionnaire tool we used has been validated in adults, but not in children. Despite this limitation, the questions we included overlap with those previously used in instruments validated for children.^{13,40} Unlike instruments specific for pediatric populations,¹³ we are missing items assessing experiences unique to pediatric populations and as a result are likely underestimated the true prevalence of discrimination and magnitude of the association of interest in our study population. We limited our questions to those that are relevant to children and in doing so may have missed other forms of discrimination aimed at the participants or at their primary caregivers. Despite our study limitations, our study population is one of the largest pediatric African American groups with asthma. In addition, we collected a wide breadth of sociodemographic, medical history and environmental exposure data.

Future studies should aim for the development of an advanced tool to assess experiences of discrimination and other psychosocial stressors in youth. Furthermore, studies addressing discrimination across the lifespan could give better insight to how asthma outcomes change based on acute versus chronic psychosocial stressor exposure and allow interventions to take place with follow up to examine changes in asthma outcomes. Finally, other asthma endotypes such as atopic asthma and obese asthma would be worth examining to observe their response to psychosocial stress. Strengthening risk profiling abilities will allow health care providers to identify those at highest risk and intervene earlier in children's lives, when they are most susceptible to social stress.

CONCLUSION:

Our study confirms previous findings that psychosocial stress impacts asthma outcomes in children.^{3,11} We found BDR to be increased in participants reporting discrimination and that this association was limited to African American youth with TNF- α high asthma, an asthma type thought to be resistant to traditional asthma medications. This is clinically important, as those who are at risk of poor asthma outcomes and were previously thought to be unresponsive to asthma medications¹⁷ may actually be responsive and may benefit from adjunct behavioral or environmental interventions. These results support the need to screen for psychosocial stress among those with moderate-severe asthma as it may reclassify asthma type and identify more precise treatments for those at high-risk.

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“Neeta Thakur and Esteban Burchard had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.”

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Tables

Table 1: Selected Characteristics of Participants with Asthma in SAGE II (2006-2014).

Characteristic	Discrimination - None	Discrimination - Any	p -value
	No. (%) [*]	No. (%) [*]	
Prevalence	295 (51.2)	281 (48.8)	
Age , median (IQR)	12.1 (4.8)	15.4 (5.5)	< 0.001
Sex , male	160 (54.2)	151 (53.7)	0.904
Tobacco Exposure			
Current	82 (28.4)	88 (31.5)	0.410
In-Utero	45 (15.3)	62 (22.1)	0.036
Daycare Attendance			
Yes	204 (69.2)	208 (74.0)	0.196
No	91 (30.8)	73 (26.0)	
Education Level[^]			
Some HS [†]	35 (11.9)	32 (11.4)	0.008
HS Graduate	98 (33.2)	60 (21.4)	
Some College	162 (54.9)	189 (67.3)	
%African Ancestry , mean (SD)	77.3 (12.7)	78.9 (11.0)	0.298
Atopy			
None	104 (35.9)	106 (38.0)	0.976
Rhinitis or Eczema	119 (41.0)	102 (36.6)	
Both	67 (23.1)	71 (25.4)	
Asthma Control			
Controlled	110 (37.3)	59 (21.0)	< 0.001
Not well Controlled	85 (28.8)	81 (28.8)	
Very Poorly Controlled	100 (33.9)	141 (50.2)	
Controller medication use			
No	178 (60.3)	190 (67.6)	0.069
Yes	117 (40.0)	91 (32.4)	
TNF-α level			
High	142 (48.1)	136 (48.4)	0.950
Low	153 (51.9)	145 (51.6)	
% Bronchodilator Response , mean (SD)	8.9 (7.8)	10.8 (9.4)	0.006

Definition of Abbreviations: HS = high school, SAGEII = Study of African Americans, Asthma, Genes and Environment, SES = socioeconomic status

^{*}Values are reported as numbers (percentages) unless otherwise specified

[^]Refers to the education level of the participant's mother

[†] Discrimination was categorized as Never (negative answer to all 4 situations); Any (affirmative answer to one or more situations)

Table 2: Mean Difference in Bronchodilator Response[^] and 95% CI for Reports of Racial Discrimination and according to TNF- α status for SAGE II Participants with Asthma (2006-2014)

		TNF- α Status ²		
		Adjusted ¹	Low ¹	High ¹
Racial Discrimination				
Never	Reference	Reference	Reference	Reference
Any	1.70 (0.36, 3.03)	0.78 (-1.07, 2.63)	2.78 (0.79, 4.77)	

[^] Bronchodilator response: mean percentage change in measured FEV₁ before and after albuterol administration, using the post-albuterol spirometry with the maximal change.

¹adjusted for sex, age, maternal education, recruitment center, *in utero* smoke exposure, daycare attendance, baseline lung function, controller medication use, African ancestry, TNF- α mean, and biomarker storage time.

²p-interaction = 0.04

Online Supplemental

Text Results:

High versus Low TNF- α asthma

Selected characteristics of participants with low and high TNF- α are displayed in **Table E2**. When compared with youth with low TNF- α , participants with high TNF- α were younger (median age 12.8 versus 14.1 years, $p=0.012$), more likely to be male (62.2 versus 46.3%, $p<0.001$) and more likely to have very poorly controlled asthma (47.1 versus 36.9%, $p=0.018$).

Figure Legend:

eFigure 1. Bronchodilator response (%) by level of reported perceived discrimination (None/Any) stratified by TNF- α status for participants with asthma from SAGE II recruited from 2006-2014. Means are adjusted for sex, age, maternal education, recruitment center, *in utero* smoke exposure, daycare attendance, baseline lung function, controller medication use, African ancestry, and biomarker storage time.

Supplemental Tables

eTable 1. Eligibility Criteria for Participation for SAGE II Asthma Cases.

Criterion	Asthma Cases
Age between 8 and 21 years old	Yes
All four grandparents self-identified as African American (SAGE II)	Yes
History of physician-diagnosed asthma	Yes
Symptoms of wheezing or shortness of breath	Yes
No respiratory infections for ≥ 6 weeks (clinical stability)	Yes
No asthma exacerbations for ≥ 6 weeks (clinical stability)	Yes
Less than 10 pack year smoking history and no smoking in the last year	Yes
If pregnant, < 3rd trimester	Yes
No history of other lung diseases or other chronic illnesses	Yes

eTable 2: Selected Characteristics* of Participants by TNF- α status in SAGE II.

	Low TNF- α	High TNF- α	p-value
Prevalence	298 (51.7%)	278 (48.3)	
Age , median (IQR)	14.1 (6.2)	12.8 (5.4)	0.012
Sex , male	138 (46.3)	173 (62.2)	0.001
Tobacco Exposure			
Current	82 (27.7)	88 (32.4)	0.227
In-Utero	49 (16.4)	58 (20.9)	0.173
Daycare Attendance			
Yes	215 (72.1)	197 (70.9)	0.733
No	83 (27.9)	81 (29.1)	
Education Level[^]			
Some HS [†]	30 (10.1)	37 (13.3)	0.092
HS Graduate	77 (25.8)	81 (29.1)	
Some College	191 (64.1)	160 (57.6)	
Discrimination[†]			
Never	153 (51.3)	142 (51.1)	0.950
Any	145 (48.7)	136 (48.9)	
%African Ancestry , mean (SD)	77.6 (12.6)	78.6 (11.2)	0.930
Atopy			
None	97 (33.2)	113 (40.8)	0.094
Rhinitis or Eczema	120 (41.1)	101 (36.5)	
Both	75 (25.7)	63 (22.7)	
Controller medication use⁹			
No	190 (63.8)	178 (64.0)	0.946
Yes	108 (36.2)	100 (36.0)	
Asthma Control			
Controlled	96 (32.2)	73 (26.3)	0.018
Not well Controlled	92 (30.9)	74 (26.6)	
Very Poorly Controlled	110 (36.9)	131 (47.1)	
% Bronchodilator Response , mean (SD)	9.3 (8.1)	10.4 (9.1)	0.013

Definition of Abbreviations: HS = high school, SAGEII = Study of African Americans, Asthma, Genes and Environment

*Values are reported as numbers (percentages) unless otherwise specified

[^]Refers to the education level of the participant's mother

[†]Discrimination score was categorized as Never (negative answer to all 4 situations); Any (affirmative answer to one or more situations)