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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 addressed the cause, treatment, and prevention of asthma in high-risk populations. This was achieved by delineating the pathways by which social adversities impart their effects on asthma susceptibility and morbidity in minority populations. Aim 1 focused on the immune system and hypothalamus-pituitary-adrenal axis response to social adversities and their effect on asthma susceptibility and morbidity. We have measured 1000 of the proposed total of 1000 samples. Aim 2 focused 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) has been completed. This proposal provided avenues to better identify high-risk populations, which will influence the development of interventions that target the modifiable aspects of social adversities to effectively improve asthma outcomes.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

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

3. OVERALL PROJECT SUMMARY:

There were two major goals for the study that aligned with the Specific Aims. The **first major goal** was to measure biomarkers related to the immune and neuroendocrine system. The **second major goal** was to measure and examine the oral microbiome in relation to measures of psychosocial and socioeconomic stress and asthma. Of note, the proposed timeline for our project was delayed, which has had an overall impact of delaying the analysis phase of the study. We experienced a total delay of approximately 9 months (initial delay with awaiting HRPO approval, second delay with Clinical Lab testing). During the award period we have been able to complete biomarker testing on 1000 participants and measure the microbiome in approximately 200 participants. This work has resulted in improving our understanding of the relationship between adversity and asthma and has identified potential opportunities for intervention. We are actively analyzing the data created under this award and will continue to do so outside the award period. Thus far, this award has resulted in 3 manuscripts (1 published, 2 close to submission) and is anticipated to continue to contribute to scientific discovery. Moreover, the results generated under this award have directly contributed to further funding: NHLBI K23 Award (PI Thakur) and private funding from the TARA Health Foundation and the Koret Foundation ($1.5 million in directly related funding).

Under the **first major goal**, we measured immune- and neuroendocrine-related biomarkers on 1000 participants. To better understand the interaction between exposure to social adversity and biological dysregulation (toxic stress), we examine these data using the following approach (**figure 1**): first, we examined the association between social adversities (socioeconomic status, discrimination, *in utero* smoke exposure, and acculturation in our Latino population) and asthma; second, we examined the association between social adversities and toxic stress, which was measured by developing an allostatic load index that accounts for dysregulation across biologic systems, third, we examined for an association between allostatic load and asthma; and, lastly, we examined if the associations found between the social adversities tested and asthma are partially mediated through allostatic load. To examine for mediation, we used the mediation package in R (version 3.5.0) In addition to examining for mediation, we also examined whether children with high allostatic load were more susceptible to the effects of social adversity compared with children with low allostatic load; potentially, identifying a high-risk profile that may help identify children at risk and likely to benefit from intervention.

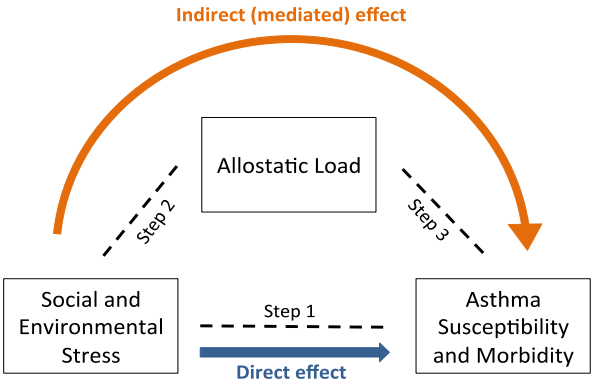

Figure 1. Summary of Analytic Approach.

Baseline Characteristics

998 children with and without asthma from SAGEII and GALA II were included in the present study. Across both studies, children with asthma were younger. In GALA II, children with asthma were more likely to male, have mothers who completed high school, and have a higher composite socioeconomic (SES) index compared with children without asthma. In SAGE II, children with asthma were less likely to be of High socioeconomic status (SES) but more likely to have private health insurance (most commonly through caregiver employer). (**Table 1**). Toxic stress is thought to be stress that is unwavering and in the presence of no buffering resilience strategy (e.g. a supportive, loving parent). It is thought that this unwavering stress leads to dysregulation across biologic systems and that leads the individual to be even more susceptible to social and environmental stressors and makes one more susceptible to disease. This dysregulation across biologic systems can be summed as an allostatic load index. Allostasis refers to the 'wear and tear' the body experiences, and, for this study, was measured using the following biomarkers: body mass index (BMI), interleukin (IL)-6, c-reactive protein (CRP), high density lipid (HDL), total cholesterol (chol), and triglycerides (trig). We were unable to assess for glucose metabolism dysregulation as glucose levels degrade overtime in frozen samples (82% study sample had random glucose levels <60 mg/dl). For BMI, obese patients were identified as a BMI percentile >95% predicted for age. For remaining biomarkers, the highest quartile for each biomarker (except HDL, which was reverse coded), was allocated a score of 1. The allostatic load index was then summed across these six variables and range from 0 to 5 (highest possible value = 6). 18% of participants were missing data for no more than 1 variable used to construct index. In **Table 1**, participants with asthma were more likely to be obese regardless of study. SAGEII participants with asthma were more likely to have lower HDL and total

cholesterol levels compared with children without asthma. GALA II participants were more likely to have higher triglyceride levels and a higher calculated allostatic load index compared with children without asthma.

Table 1. Selected Characteristics of SAGE II and GALA II Participants with and without asthma

Characteristic	**SAGEII Asthma**	**SAGE II Healthy Control**	**GALA II Asthma**	**GALA II Healthy Control**
N	341	156	263	238
Age, median (IQR)	**13.5 (6.1)**	**16.6 (6.0)**	**12.6 (5.0)**	**14.2 (6.3)**
Sex, Male, n (%)	172 (50.4)	71 (45.5)	**150 (57.0)**	**101 (42.4)**
Maternal Education, n (%)				
Grade 6-8	12 (3.5)	5 (3.2)	**94 (35.7)**	**113 (47.5)**
Grade 9-11	39 (11.4)	17 (10.9)	**53 (20.1)**	**53 (22.3)**
High School Grad+	290 (85.0)	134 (85.9)	**116 (44.1)**	**72 (30.3)**
Income Tertile, n (%)				
Low	**133 (39.0)**	**69 (44.2)**	111 (42.2)	120 (50.4)
Mid	**119 (34.9)**	**33 (21.1)**	86 (32.7)	63 (26.5)
High	**89 (26.1)**	**54 (34.6)**	66 (25.1)	55 (23.1)
Insurance, n (%)				
Uninsured	**10 (2.9)**	**13 (8.3)**	18 (6.8)	27 (11.3)
Government	**165 (48.4)**	**83 (53.2)**	187 (71.1)	172 (72.3)
Private	**166 (48.7)**	**60 (38.4)**	58 (22.0)	39 (16.3)
SES Index*, median (IQR)	7 (2)	7 (2)	**6 (2)**	**5 (2)**
SES Index, High, n (%)	146 (42.8)	59 (37.8)	59 (22.4)	38 (16.0)
In Utero Smoke, n (%)	70 (20.5)	24 (15.4)	10 (3.8)	5 (2.1)
Discriminatory Experiences				
Any, n (%)	143 (41.9)	47 (30.1)	60 (22.8)	53 (22.3)
Missing, n (%)	60 (17.6)	50 (32.1)	24 (9.1)	21 (8.8)
Acculturation, n(%)				
1st Generation	---	---	**31 (11.8)**	**73 (30.7))**
2nd Generation			**184 (70.0)**	**143 (60.1)**
3rd Generation			**48 (18.3)**	**22 (9.2)**
Adversity^, n (%)				
None	59 (21.0)	22 (20.8)	39 (16.3)	27 (12.4)
One	120 (42.7)	55 (51.9)	149 (62.3)	143 (65.9)
2 or more	102 (36.3)	29 (27.6)	51 (21.3)	47 (21.7)
Allostatic Load Indicators				
Obese, yes, n (%)	**109 (33.0)**	**23 (17.4)**	**100 (39.4)**	**24 (24.2)**
Missing, n (%)	11 (3.2)	24 (15.4)	9 (3.4)	139 (58.4)
IL-6, pg/ml median (IQR)	5.58 (49.6)	6.63 (78.8)	20.65 (165.3)	14.44 (117.55)
CRP, mg/L median (IQR)	0.39 (1.07)	0.44 (1.77)	1.84 (1.46)	2.24 (1.54)
HDL, mg/dL mean (sd)	**56.2 (15.4)**	**63.0 (17.6)**	54.7 (15.3)	54.1 (14.3)
Total Cholesterol, mg/dL, mean (sd)	**153.2 (38.0)**	**161.5 (41.2)**	155.1 (38.1)	151.6 (38.5)
Missing, n (%)	0 (0)	0 (0)	1 (0.4)	0 (0)
Triglycerides, mg/dL, median (IQR)	52.0 (37.0)	50 (26.3)	**94 (77.0)**	**78.0 (52.0)**
Missing, n (%)	1 (0.3)	0 (0)	2 (0.7)	0 (0)
Allostatic Load Index, median (IQR)	1 (2)	1 (1)	**2 (2)**	**1 (1)**
Allostatic Load Index, 2+, n(%)	100 (29.3)	38 (24.4)	**132 (50.2)**	**78 (32.8)**
Asthma Outcomes				
FEV1 % predicted, mean (sd)	98.5 (14.1)	----	97.9 (13.4)	----
Missing, n (%)	22 (6.5)		6 (2.3)	
Bronchodilator Response, %change, mean (sd)	9.65 (7.50)	----	8.00 (6.70)	----
Missing, n (%)	22 (6.5)		6 (2.3)	

Bolded values indicate P<0.05. *Composite SES Index includes household income, maternal education, and insurance type. ^Adversity Composite variable includes SES, discriminatory experiences, and in utero tobacco exposure

Social Adversities and Asthma

We previously demonstrated that socioeconomic status is inversely associated with asthma in African American children and that this relationship is most pronounce in children with non-atopic asthma (Thakur AJRCCM 2013). In this same study, we demonstrated that higher SES was associated with asthma in Mexican American children and that this relationship may be partially attenuated with acculturation. In the current study, which is a subset of participants from this parent study, we do not observe a relationship between SES and asthma in African American children; however, we see a similar association between high SES and asthma in our GALA II Participants (all included self-identify as Mexican American) (**Table 2**). It is not surprising that we no longer see an association between low SES and asthma in our African American population as high SES and healthy control participants in SAGEII (our African American study) were more likely to give saliva samples (as oppose to whole blood) compared with those of low SES and with asthma. This systematic bias in sample collection has likely biased our results towards the null. We have also demonstrated that *in utero* smoke exposure was associated with asthma and worse asthma control in SAGEII and GALAII (Oh JACI 2012). We observe a similar association, albeit non-significant, in the present study.

Since funding of this award, we have demonstrated that discriminatory experiences are associated with asthma and worse asthma outcomes in African American children and among low SES Mexican American children (Thakur Chest 2017). In the present study, we observed that any reported experience of racial/ethnic discrimination was associated with asthma in SAGEII (**Table 2**). Most recently, we demonstrated that acculturation was associated with asthma and pulmonary function in Latino patients (Thakur JACI 2019). A similar association with asthma was observed in the present study (**Table 2**).

Table 2. Association between Select Social Adversities and Asthma in SAGEII and GALAII Participants

Exposure		Total Population OR (95% CI)	SAGE II OR (95% CI)	GALA II OR (95% CI)
SES Score (low to high)	Unadjusted	**1.27 (1.16, 1.38)**	1.09 (0.95, 1.25)	**1.29 (1.12, 1.48)**
	Adjusted*	**1.22 (1.10, 1.35)**	1.01 (0.95, 1.27)	**1.35 (1.17, 1.57)**
In Utero Smoke Exposure	Unadjusted	1.92 (1.24-3.04)	1.42 (0.87-2.40)	1.84 (0.64-5.99)
	Adjusted*	1.55 (0.97-2.53)	1.53 (0.91-2.64)	1.61 (0.54-5.43)
Experiences of Discrimination, Any	Unadjusted	**1.37 (1.02-1.85)**	1.21 (0.77-1.90)	1.04 (0.68-1.59)
	Adjusted*	**1.50 (1.07-2.11)**	**1.68 (1.04-2.74)**	1.34 (0.83-2.17)
Acculturation				
1st Generation		---	---	Reference
2nd Generation	Unadjusted			**3.03 (1.90, 4.92)**
	Adjusted*	---	---	**2.74 (1.69, 4.51)**
3rd Generation	Unadjusted			**5.14 (2.69, 10.07)**
	Adjusted*	---	---	**4.20 (2.15, 8.42)**

*Total Population models adjusted for Age, Sex, Study, and Region. SAGEII model adjusted for Age and Sex. GALAII model adjusted for Age, Sex, and Region.

Asthma, Social Adversity and Allostatic Load

Across the two studies, children with asthma had higher allostatic load indices compared with children without asthma (1.09 vs. 1.46, **Table 3**). In the total study population, we demonstrated that high SES was associated with a 0.19 decrease in the allostatic load index compared with children with low SES (95%CI -0.35, -0.03). When stratified by study, this association remained

statistically significant for SAGE II participants. We also observed that participants with *in utero* smoke exposure, on average, had a 0.24 increase in their allostatic load index compared with children with no history of *in utero* smoke exposure. No association was seen between the allostatic load index and experiences of discrimination. In our GALA II participants, we observed a 0.33 and 0.40 increase in allostatic load for second generation and third generation participants, respectively, compared with first generation participants. (**Table 3**)

Table 3. Estimate Effect of Asthma and Select Social Adversities on Allostatic Load in SAGEII and GALAII Participants

		Total Population β coef (95% CI)	SAGE II β coef (95% CI)	GALA II β coef (95% CI)
Outcome				
Asthma	Unadjusted	**0.28 (0.14, 0.43)**	**0.21 (0.01, 0.40)**	**0.52 (0.31, 0.73)**
	Adjusted*	**0.37 (0.22, 0.52)**	**0.24 (0.04, 0.45)**	**0.46 (0.25, 0.67)**
Exposure				
SES Score, high	Unadjusted	**-0.33 (-0.49, -0.18)**	**-0.21 (-0.39, -0.02)**	-0.25 (-0.51, 0.02)
	Adjusted*	**-0.19 (-0.35, -0.03)**	**-0.21 (-0.40, -0.03)**	-0.16 (-0.44, 0.12)
In Utero Smoke Exposure	Unadjusted	0.02 (-0.21, 0.24)	0.19 (-0.04, 0.42)	0.44 (-0.18, 1.06)
	Adjusted*	**0.24 (0.01, 0.47)**	0.18 (-0.05, 0.42)	0.39 (-0.22, 1.00)
Experiences of Discrimination, Any	Unadjusted	-0.16 (-0.33, 0.00)	-0.05 (-0.26, 0.16)	-0.06 (-0.32, 0.21)
	Adjusted	-0.08 (-0.25, 0.10)	-0.09 (-0.31, 0.12)	-0.07 (-0.34, 0.21)
Acculturation				
1st Generation		---	---	Reference
2nd Generation	Unadjusted			**0.38 (0.11, 0.64)**
	Adjusted	---	---	**0.33 (0.06, 0.59)**
3rd Generation	Unadjusted			**0.43 (0.06, 0.79)**
	Adjusted	---	---	**0.40 (0.02, 0.77)**

*Total Population models adjusted for Age, Sex, Study, and Region. SAGEII model adjusted for Age and Sex. GALAII model adjusted for Age, Sex, and Region.

Mediation Analysis

Based on the presented results, we carried out two mediation analysis to examine whether the association 1) between socioeconomic status and asthma in the total population and 2) between acculturation and asthma in the GALAII population is partially mediated through allostatic load. The association between SES and asthma in the total population was partially mediated by allostatic load. Among GALAII participants, a small proportion of the total effect of acculturation on asthma was mediated through allostatic load (**Table 4**).

Table 4. Mediator Effects of Allostatic Load in the Associations between Select Social Adversities and Asthma

	Estimate (95% CI)	p-value
Socioeconomic Status*		
ACME	-0.014 (-0.026, 0)	0.006
ADE	0.091 (0.026, 0.16)	0.007
Total Effect	0.076 (0.011, 0.14)	0.02
Proportion Mediated	-0.18 (-1.07, -0.03)	0.03
Acculturation^		
ACME	0.026 (0.003, 0.06)	0.02
ADE	0.30 (0.16, 0.45)	<0.001
Total Effect	0.33 (0.19, 0.47)	<0.001
Proportion Mediated	0.08 (0.01, 0.21)	0.02

Abbreviations: ACME: Average causal mediation effect; ADE: average direct effect; *In SAGEII and GALAII combined; ^In GALAII

Asthma Phenotypes and Social Adversity

Asthma is now thought of as a heterogenous disorder composed of several clinical phenotypes with different risk factors. The phenotypes are a first attempt to categorize high-risk patients and predict outcomes, such as corticosteroid responsiveness. However, current methods fail to incorporate social and environmental data into the development of phenotypes. The incorporation of these important risk factors may re-classify disease type and change treatment options for patients. We found that TNF-alpha, a pro-inflammatory cytokine associated with both asthma and psychosocial stress, to be an important biomarker to differentiate controlled from not well controlled asthma, especially when combined with report of discriminatory experiences (a potent psychosocial stressor) in our African American participants with asthma. For this study, we examined 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 TNF-alpha high and low asthma (phenotypes of asthma).

Almost half of participants (48.8%) reported experiencing racial discrimination. Those reporting discriminatory experiences 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 1**). 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 presented as an oral abstract at the 2016 UCSF Health Disparities Forum (San Francisco, CA), the 2017 American Thoracic Society meeting (Washington, D.C.) and published in PLOS One as a scientific manuscript (Carlson PLOS One 2017).

Table 1: 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 FEV_1 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

Among the 1000 participants with measured biomarkers, 689 had complete data for socioeconomic status, experiences of discrimination, and early-life NO_2 exposure (a marker for traffic-related air pollution). These three variables represent different aspects of adversity:

socioeconomic, psychosocial, and environmental stress. From these three exposure variables, a composite adversity variable was developed. Participants with 2 or more exposures were consider to have high adversity exposure and those with 1 or less exposure were considered to have low adversity exposure. CCL17 (thymus and activation regulated chemokine-TARC) is produced in the thymus by dendritic cells and binds to a region on Th2 lymphocytes and induce an allergic response (elevated in Th2 high asthma— a well-defined atopic endotype of asthma). Among children without asthma, participants with the high adversity were more likely to have elevated CCL17/TARC compared with those with low adversity (94.0 pg vs 71.8 pg, p=0.06); no difference in CCL17/TARC levels were noted among kids with asthma by adversity exposure. Similarly, we found that CHI3L1 (YKL-40), a cytokine elevated in asthma, was elevated in healthy controls with adversity exposure in comparison with children without adversity p=0.03) and no difference was observed among children with asthma. These findings suggest that adversity exposures are associated with increased atopy response in children without asthma. We may not observe a similar response in children with asthma due to an overall increase due to the underlying disease state versus a differential based on case/control status. These findings are being prepared as a manuscript and the association is being further explored by examining the association between adversity exposure with allergen sensitization patterns using skin-prick data, the gold standard for confirming positive atopy response.

The **second major goal** under this award was to measure and examine the oral microbiome in relation to measures of psychosocial and socioeconomic stress and asthma. We have deep sequenced the 16s rRNA gene from the DNA in a total of 249 saliva samples from participants (188 supported from this award). DNA was obtained from saliva samples collected in Oragene DNA Self Collection kits following manufacturer's instructions. We compared the measured V4 region from our samples to recorded libraries to define the oral microbiome in terms of richness, diversity and bacterial taxonomy. Sequencing was performed in a MiSeq sequencer. Relative abundance was calculated at phylum and genus levels separately. Differences in the abundance of each phylum and genus were assessed using the non-parametric Wilcoxon test and adjustment for multiple comparisons was carried out via Bonferroni correction for the number of genera tested. The characteristics of the participants sequenced is **Table 4.**

Table 4. Characteristics of Participants with Measured Microbiome Data

	African American			**Latinos**		
	Case	Control	*p*-value	Case	Control	*p*-value
	n = 57	n =57		n= 33	n=102	
Sex (% female)	49.1% (28)	63.2% (36)	0.1864	48.5% (16)	56.9% (58)	0.523
Age	15.6± 3.3	15.0±3.9	0.493	11.1±2.7	12.6±2.8	0.005
African (%)	83.3±7.6	75.6±14.0	<0.001	15.9±13.6	15.7±11.2	0.824
European (%)	16.7±7.6	24.4±14.0	<0.001	57.2±17.5	57.0±18.6	0.734
Native American (%)	--	--	--	26.9±23.5	27.3±25.2	0.707
FEV_1 (% predicted)	94.8±13.1	NA	--	90.9±17.5	NA	NA
FVC (% predicted)	102.0±14.5	NA	--	94.2±17.1	NA	NA

The salivary microbiome from Latino cases and controls was composed by five phyla (Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria), that together accounted for ~99% of the reads. None of the Phyla presented differences between groups. At genus level, on average 45 genera were detected, of which 17 genera had a relative abundance greater than 1%. The most abundant genera were Prevotella, Streptococcus, Haemophilus, and

Veillonell No differences were found between the diversity of cases and controls within our Latino population (**Table 5)**.

Table 5. Summary of the comparison of genera mean relative abundance (%) among the Latino Participants by case/control status.

Taxa	Cases	Controls	*p*-value	Bonferroni *p*-value
Actinobacillus	1.25	1.13	0.96	1
Actinomyces	3.08	2.38	0.11	1
Aggregatibacter	2.16	2.24	0.57	1
Atopobium	0.92	1.14	0.32	1
Campylobacter	0.98	1.19	0.3	1
Fusobacterium	4.14	3.72	0.57	1
Granulicatella	1.12	0.65	0.044	0.792
Haemophilus	12.82	14.48	0.43	1
Leptotrichia	1.82	1.7	0.12	1
Megasphaera	0.94	1.34	0.32	1
Neisseria	5.62	5.95	0.77	1
Oribacterium	0.96	1.03	0.91	1
Porphyromonas	1.66	1.68	0.67	1
Prevotella	18.39	20.84	0.15	1
Rothia	4.46	4.04	0.5	1
Streptococcus	18.79	15.64	0.086	1
Veillonella	8.74	9.23	0.58	1

Statistically significant *p*-values are shown in bold.

Case and control samples collected from our African American participants contained five major bacterial phyla (Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria, and Fusobacteria) that accounted for more than 98% of the sequence reads. At the genus level, the salivary microbiome contained an average 122 of 56 genera, of which 15 had a relative abundance greater than 1% in cases or controls. The most abundant genera were Prevotella, Haemophilus, Streptococcus, and Veillonella. At the phylum level, no differences were found between cases and controls. However, statistically significant differences in relative abundance at the genus level were found for Streptococcus (13.0% in cases and 18.3% in controls; $P = 2.6 \times 10^{-3}$, Bonferroni P = .039) and Veillonella (11.1% in cases and 8.0% in controls; $P = 2.3 \times 10^{-3}$, Bonferroni P = .035) (**Table 6**).

Table 6. Summary of the comparison of genera mean relative abundance (%) among the African American Participants by case/control status.

Taxa	Cases	Controls	*p*-value	Bonferroni *p*-value
Actinomyces	3.08	2.38	0.31	1
Aggregatibacter	2.16	2.24	9.8×10^{-3}	0.147
Atopobium	0.92	1.14	0.3049	0.735
Campylobacter	0.98	1.19	0.68	1
Fusobacterium	4.14	3.72	0.98	1
Haemophilus	12.82	14.48	0.89	1
Leptotrichia	1.82	1.7	0.67	1
Megasphaera	0.94	1.34	0.049	0.735
Neisseria	5.62	5.95	0.074	1
Oribacterium	0.96	1.03	0.37	1
Porphyromonas	1.66	1.68	0.22	1
Prevotella	18.39	20.84	0.67	1
Rothia	4.46	4.04	0.38	1
Streptococcus	18.79	15.64	2.6×10^{-3}	**0.039**
Veillonella	8.74	9.23	2.3×10^{-3}	**0.035**

Statistically significant *p*-values are shown in bold.

With the current sample size, we did not observe any associations between SES and diversity using the Shannon and the Pielou diversity indices. Therefore, we did not meet a priori criteria to complete a mediation analysis (**Figure 1,** exposure is associated with outcome [step 1), exposure is associated with mediator [step 2], outcome is associated with mediator [step 3]). However, others have demonstrated that socioeconomic status is associated with microbiome differences and that these differences may contribute to asthma morbidity (Levin Scientific Reports 2016). One main difference is that these studies included gut microbiome and not salivary samples. To increase our power to assess for differences according to psychosocial stress exposures, we plan to sequence additional samples to examine the microbiome in an additional subset of the GALAII and SAGEII population (n=96). This will include examining for differences in diversity using the Shannon and the Pielou diversity indices and to explore associations with other psychosocial stress measures.

KEY RESEARCH ACCOMPLISHMENTS:

- Concomitant consideration of biologic and psychosocial stressor measures identifies at-risk asthmatics. We demonstrated that the addition of a psychosocial measure, experiences of discrimination, help identify children who may benefit from further intervention among those with a difficult to control asthma phenotype (TNF-alpha high asthma). 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.

- Socioeconomic status and adversity are associated with atopic and non-atopic pathways in children with and without asthma. Specifically, low SES is associated with indoor allergen sensitization, which may offer an important point for intervention to reduce risk of asthma later in life and allow for better asthma control in those with asthma already.

4. CONCLUSION:

This study takes us a step closer to understanding risk factors for asthma in low-income, minority communities. We demonstrated how the consideration of psychosocial stressors and biomarkers 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. We also demonstrated that SES operates through two mechanisms: 1) non-atopic pathways and 2) sensitization to indoor allergens. These patterns should be further explored in other cohorts of inner-city asthma and in a longitudinal study. They also offer a potential point of intervention at the community level- targeted efforts for mold and rodent control. We are actively analyzing the data created under this award and will continue to do so outside the award period; specifically, we will complete a manuscript describing the association of allostatic load and asthma and a manuscript reporting the results of the microbiome and asthma study.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

(1) Lay Press:

- Leigh, S. **Racism Aggravates Treatment-Resistant Asthma.** UCSF News Center. June 14, 2017. (https://www.ucsf.edu/news/2017/06/407361/racism-aggravates-treatment-resistant-asthma)

(2) Peer-Reviewed Scientific Journals:

- S. Carlson, Borrell N, Eng C, Nguyen M, Thyne S, LeNoir MA, Burke-Harris N, Burchard EG*, Thakur N*. *These authors contributed equally to this work. Self-reported racial/ethnic discrimination and bronchodilator response in African American youth with asthma. PLoS ONE 12(6): e0179091. PMID 28609485

(3) Abstracts:

- 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. (Oral Presentation)
- N Thakur, 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.

Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma. American Journal of Respiratory and Critical Care Medicine; New York Vol. 195, (2017): 1 (Oral Presentation)

b. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

- N Thakur, 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. Perceived Discrimination Affects Bronchodilator Response in African American Youth with Asthma. American Journal of Respiratory and Critical Care Medicine; New York Vol. 195, (2017): 1 (May 2017 Oral Presentation)*
- Asthma Forum. "Discrimination, Psycho-Social Stress and the Relationship to Non-Atopic Neutrophilic Asthma and Other Asthma Phenotypes", Kaiser Permanente, San Francisco, California. (November 2017)
- Koret Institute for Precision Prevention. "A Multilevel Approach to Understanding Asthma Health Disparities", UC Berkeley. Berkeley, California. (December 2017)

5. INVENTIONS, PATENTS AND LICENSES:
Nothing to report

6. REPORTABLE OUTCOMES:
Nothing to report

7. OTHER ACHIEVEMENTS:

The 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. This study also led to award funding to UCSF (PI: Thakur) from the Koret Foundation (Sub-contract from UC Berkeley) to establish a longitudinal cohort of children with and without asthma to better understand the relationship between atopy, adversity, and asthma. In both studies, 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.

10. REFERENCES:

S. Carlson, Borrell N, Eng C, Nguyen M, Thyne S, LeNoir MA, Burke-Harris N, Burchard EG*, Thakur N*. *These authors contributed equally to this work. Self-reported racial/ethnic discrimination and bronchodilator response in African American youth with asthma. PLoS ONE 12(6): e0179091. PMID 28609485

11. 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, TARA Health
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

ACTIVE

Parker B. Francis Fellowship Program 7/1/2015-6/30/2018 4CM
Francis Family Foundation 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

W81XWH-15-1-0116 (Thakur) 09/31/2015-06/29/2018 2.4CM
Discovery Award/DOD 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
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

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

- **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.

TRAINING OR FELLOWSHIP AWARDS:
Not applicable

COLLABORATIVE AWARDS:
Not Applicable

QUAD CHARTS:
Not Applicable

MARKING OF PROPRIETARY INFORMATION:
"Approved for Public Release"

12. APPENDIX

Self-reported racial / ethnic discrimination and bronchodilator response in African American youth with asthma. PLoS ONE 12 (6): e0179091. PMID 28609485

PLOS ONE

RESEARCH ARTICLE

Self-reported racial/ethnic discrimination and bronchodilator response in African American youth with asthma

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Data Availability Statement: Due to the sensitive nature of the data collected surrounding issues of race, discrimination, nativity status, and ancestry, data are available upon request. Access to the limited dataset used in this study can be arranged by contacting Dr. Esteban Burchard [email: esteban.burchard@ucsf.edu]. Of note, any data provided will be stripped of specific subject identifiers that could be used to identify a specific child and/or his/her residence.

Abstract

Importance

Asthma is a multifactorial disease composed of endotypes 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 racial/ethnic discrimination.

Objective

We examined the association between self-reported racial/ethnic discrimination and bronchodilator response (BDR) among African American youth with asthma ages 8 to 21 years (n = 576) and whether this association varies with tumor necrosis factor alpha (TNF-α) level.

Materials and methods

Self-reported racial/ethnic discrimination was assessed by a modified Experiences of Discrimination questionnaire as none or any. Using spirometry, BDR was specified as the mean percentage change in forced expiratory volume in one second before and after albuterol administration. TNF-α was specified as high/low levels based on our study population mean. Linear regression was used to examine the association between self-reported racial/ethnic discrimination and BDR adjusted for selected characteristics. An interaction term between TNF-α levels and self-reported racial/ethnic discrimination was tested in the final model.

Funding: This work was supported in part by the Sandler Family Foundation, the American Asthma Foundation, the RWJF Amos Medical Faculty Development Program, Harry Wm. and Diana V. Hind Distinguished Professor in Pharmaceutical Sciences II, Center for Youth Wellness, National Institutes of Health 1R01HL117004, R01 ES015794, R01HD085993, National Institute of Health and Environmental Health Sciences R21ES24844, the National Institute on Minority Health and Health Disparities 1P60 MD006902 and 1R01MD010443; Tobacco-Related Disease Research Program under Award Number 24RT-0025; Department of Defense (PR141896); N.T. was supported by career development awards from the NHLBI (K12-HL119997 and K23-HL125551-01A1), Parker B. Francis Fellowship Program, and the American Thoracic Society. S.C. was supported through the UCSF PROF-PATH program by R25MD006832 from the NIMHD. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: NB-H is currently the CEO of the Center for Youth Wellness, a funder of this study. The Center for Youth Wellness paid for the assays and supplies used to measure biomarkers in SAGE II participants. This does not alter our adherence to PLOS ONE policies on sharing data and materials. All other authors have declared that no competing interests exist.

Results

Almost half of participants (48.8%) reported racial/ethnic discrimination. The mean percent BDR was higher among participants reporting racial/ethnic discrimination than among those who did not (10.8 versus 8.9, p = 0.006). After adjustment, participants reporting racial/ethnic discrimination had a 1.7 (95% CI: 0.36–3.03) higher BDR mean than those not reporting racial/ethnic discrimination. However, we found heterogeneity of this association according to TNF-α levels (p-interaction = 0.040): Among individuals with TNF-α high level only, we observed a 2.78 higher BDR mean among those reporting racial/ethnic discrimination compared with those not reporting racial/ethnic discrimination (95%CI: 0.79–4.77).

Conclusions

We found BDR to be increased in participants reporting racial/ethnic 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 racial/ethnic discrimination in those with asthma as it may reclassify disease pathogenesis.

Introduction

Despite asthma prevalence variation according to sex over the life course, African Americans have one of the highest asthma prevalence and mortality rates in the U.S.[1] Overall, African American children experience higher prevalence of asthma (11.2%) than non-Hispanic whites (7.7%). This is also true for asthma mortality (0.23 per 1000 individuals in African Americans versus 0.13 per 1000 individuals in non-Hispanic whites) [1]. While there are well known risk factors for these disparities, psychosocial stress [2], including experiences of racial/ethnic discrimination [3], seems to be surfacing as an important risk factor. Experiences of racial discrimination are biased treatment associated with individual characteristics such as skin color [4]. A high proportion of minority youth (up to 88%) have reported experiencing racial discrimination [5].

However, the response to psychosocial stress is inconsistent [6,7] and, similarly, may also vary with experiences of racial/ethnic discrimination as the result of the heterogeneous nature of asthma. Asthma is no longer thought of as a single disease, but as a disorder composed of distinct types with varying pathophysiology. These varying types are endotypes of asthma and are thought to reflect a particular biologic mechanism linked to specific health outcomes such as inhaled corticosteroid response and frequent exacerbations [8]. Consequently, experiences of racial/ethnic discrimination may affect asthma outcomes differently according to these asthma endotypes.

A commonly used outcome of asthma is bronchodilator response (BDR), which aids in diagnosis [9], to assess responsiveness to inhaled corticosteroids, and as a predictor of future lung function [10]. Previous research has shown that a BDR $\geq$ 10% is associated with poor asthma control. Therefore, BDR is thought to be useful as a clinical tool to identify individuals at risk of poor asthma outcomes. Youth who experience racial/ethnic discrimination tend to have poor asthma control [3,11,12]. Thus, it is important to measure and assess the effects of discriminatory experiences related to race and/or ethnicity on BDR.

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) [13]. This asthma endotype is characterized as having lower lung function [14]. It has been previously showed 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 [15]. Even within one endotype of asthma there are overlapping mechanisms [16], and thus, individuals may respond differently to the same trigger, including racial/ethnic discriminatory experiences.

Objective: We aimed to examine the association of self-reported racial/ethnic discrimination with BDR to albuterol among youth and whether this association varies with TNF-α levels.

Materials and methods

Study population

Participants for this study were enrolled through the Study of African Americans, Asthma, Genes & Environments (SAGE II) 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 II 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 [9]. To be eligible for the study, the survey respondent (participants <16 years old) or participant (≥16 years old) must have self-identified the parents and all four grandparents of the participant as African American. Those in the third trimester of pregnancy, a ≥10 pack-year smoking history, and current smokers were not eligible (S1 Table). All parents/participants provided appropriate written consent/assent. The University of California, San Francisco and each study site institutional review board (IRB) approved the SAGE II protocol (UCSF-IRB# 10–02877, Reference#155745).

Assessment of self-reported racial/ethnic 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. Interviewers were recruited from the same communities of potential participants and trained on respectively ascertaining sensitive information through questionnaires, spirometry, and biospecimen collection. The primary exposure for this analysis was self-reported racial/ethnic discrimination, ascertained using the Experiences of Discrimination (EOD) Questionnaire [17]. Consistent with a previous study [3], we included questions pertaining to our population: 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*. Experiences of discrimination were specified 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 [18]. 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 x-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. Consistent with previous studies [19,20], we classified individuals as TNF-α high and low based on being above or below our study population mean of 1.42 pg/ml.

Covariates

Informed by previous studies, age [1], sex [21], *in utero* smoke exposure [22] (i.e., maternal smoking during pregnancy), socioeconomic status [23], body mass index (BMI) [24], early life exposure to daycare [25], and African ancestry [26] were considered as potential confounders. We used maternal educational attainment as a stable measure of socioeconomic status [3] and 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 growth 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 [28].

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 force expiratory volume per one second (FEV_1) measurement with a cutoff of 80%. The brief medication questionnaire [29] was used to ascertain reported controller medications use to ascertain asthma control. The National Heart, Lung, and Blood Institute's (NHLBI) definition of asthma control is a composite score and the accepted standard to measure control [9]. Asthma control was derived from information collected through a modified version of the 1978 American Thoracic Society–Division of Lung Diseases Epidemiology Questionnaire [30] on symptoms, nighttime awakening, interferences with normal activities, and rescue medication use during the week prior to participant recruitment and interview and lung function measurements. Asthma control was defined for our analysis purposes as Controlled, Partially Controlled, or Poorly Controlled [3,22,25]. 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 considered as a covariate.

Pulmonary function measures and bronchodilator response

The primary outcome for this study was maximal BDR to albuterol. All asthma medications were held for 12 hours before spirometry. Per the American Thoracic Society recommendations, pulmonary function was measured before albuterol administration and then repeated 15 minutes after administration of four puffs of albuterol (90 μg per puff) [31]. 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) [32]. We assessed the maximal BDR as the mean percentage change in measured FEV_1 before and after albuterol administration, using the post-albuterol spirometry with the maximal change. For analytical purposes, BDR was specified as a continuous variable.

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 self-reported racial/ethnic 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 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 self-reported racial/ethnic discrimination were calculated. Significance differences and associations were determined using Student t-test and Kruskal-Wallis test according to whether continuous variables were normally distributed or not, respectively, and chi-square tests for categorical variables. Covariates associated with BDR ($p < 0.2$) were included in the final model. We used linear regression to estimate the association between self-reported racial/ethnic discrimination and BDR before and after controlling for selected covariates. To determine whether this association varies with TNF-α level, an interaction term between self-reported racial/ethnic discrimination and TNF-α was tested in the final model. Significance for main effects was determined at 0.05 and for interaction terms at 0.10. All analyses were conducted with R 3.1.2 [33].

Results

Baseline study characteristics

Selected characteristics of participants according to self-reported racial/ethnic discrimination are displayed in Table 1. Almost half (48.8%) of our participants reported experiences of racial/ethnic discrimination in any setting at some point in their life. When compared with youth who do not report experiencing racial/ethnic discrimination, participants with self-reported experiences of racial/ethnic 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 higher levels of educational attainment compared with those who did not reported racial/ethnic discrimination (67.3 versus 54.9%, p-value = 0.008). Participants who reported racial/ethnic discrimination were more likely to have very poorly controlled asthma (50.2 versus 33.9%; $p < 0.001$). Moreover, the mean percent BDR was higher among those reporting racial/ethnic discrimination (10.8, SD 9.8) than among those not reporting racial/ethnic discrimination (8.9, SD 7.8; p = 0.006). There was no association between TNF-α and self-reported racial/ethnic discrimination.

Bronchodilator response and self-reported racial/ethnic discrimination

Participants who reported any racial/ethnic discrimination had a 1.7 (95%CI 0.36–3.03) greater mean percent BDR compared with children not reporting racial/ethnic 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). However, a significant heterogeneity of this

Table 1. Selected characteristics of participants with asthma according to self-reported racial/ethnic discrimination in SAGE II (2006–2014).

Characteristic	Racial/ethnic Discrimination[a]		
	None	Any	
	No. (%)[b]	No. (%)[b]	p -value
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[c]			
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

[a] Racial/ethnic discrimination was categorized as None (negative answer to all 4 situations) or Any (affirmative answer to one or more situations)

[b]Values are reported as numbers (percentages) unless otherwise specified

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

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association was observed according to TNF-α status (High/Low; p-interaction = 0.040). For participants in the TNF-α high group, those reporting racial/ethnic discrimination had a 2.78 (95%CI: 0.79–4.77) greater mean percent BDR to albuterol than those not reporting racial/ethnic discrimination. This association was not observed among those in the TNF-α low group (S1 Fig). Selected characteristics of participants with TNF-α high and low asthma are reported in the supplement and displayed in S2 Table.

Table 2. Mean difference in bronchodilator response[a] and 95% CI for reports of racial/ethnic discrimination and according to TNF-α status for SAGE II participants with asthma (2006–2014).

		TNF-α Status[c]	
	Adjusted[b]	Low[b]	High[b]
Racial/ethnic Discrimination			
Never	0	0	0
Any	1.70 (0.36, 3.03)	0.78 (-1.07, 2.63)	2.78 (0.79, 4.77)

[a] Bronchodilator response: mean percentage change in measured FEV_1 before and after albuterol administration, using the post-albuterol spirometry with the maximal change.

[b]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.

[c]P-interaction = 0.04

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Discussion

In this study, we observed an association between self-reported racial/ethnic discrimination and mean BDR. However, the significant increased mean BDR with those self-reporting racial/ethnic discrimination was observed only among participants in the TNF-α high group. Our results corroborated previous studies suggesting that self-reported racial/ethnic discrimination as a psychosocial stressor may affect health in youth, including asthma outcomes [2,3,12]. In contrast to a previous study which showed that psychosocial stress reduces BDR [34], we observed an increase in BDR in participants with reports of self-reported racial/ethnic discrimination and TNF-α high level. This supports the theory that asthma is heterogeneous and that this heterogeneity extends to the endotypes already identified, such as TNF-α asthma [16].

Our study shows that screening for experiences of racial/ethnic discrimination, as a type of 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 [35]; however, these interventions are perceived as time and labor intensive. By identifying a risk factor profile that includes measures of racial/ethnic discriminatory experiences and inflammatory biomarkers, we may be better able to screen and identify individuals who are most susceptible to this type of 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 [8,36,37]. One pathway may involve inflammatory and neuro-endocrine mechanisms that lead to different asthma endotypes [38]. 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 [39]. Additionally, individuals reporting racial/ethnic discrimination have been shown to have elevated levels of cytokines, including TNF-α, compared with those not reporting racial/ethnic discrimination [40]. In asthma, psychosocial stress secondary to experiences of racial/ethnic discrimination may enhance airway inflammation by modulating immune cell function through hormonal pathways [38,41]. One mouse model shows how social stress potentially alters lung function: stress led to increased levels of TNF-α and decreased drug

response to inhaled corticosteroids [41]. A similar pathway has been described for TNF-α-high-asthma, which has been known to be severe and non-responsive to asthma medications [15].

Unmeasured factors related to discriminatory experiences may account for some of the association seen with bronchodilator response. Individuals experiencing racial/ethnic discrimination are likely to be from communities that are marginalized and the most affected by structural racism. This includes living in areas exposed to higher levels of indoor and outdoor air pollution, substandard housing, and exposure to community violence; all factors that are also associated with segregated neighborhoods and associated socioeconomic disadvantages. In fact, racial segregation has been independently shown to be a fundamental cause of health disparities in health [42], including asthma.[43]

There are several other limitations in the study. First, the cross-sectional design of our study limits our ability to identify causal relationships or reverse causation between our exposure and outcome. We are unable to determine if the observed relationship between asthma and reports of racial/ethnic discrimination is actually the result of asthma itself as a socially stigmatized status [44,45]. Second, we observed that the response to albuterol varied greatly in our study population and ranged from -10% (a negative response) to 105% increase from the pre-albuterol FEV_1. This variability in bronchodilator response is common [46] and similar to what has been observed in other study populations [34]. Our findings of increased bronchodilator response in those with high TNF-α high asthma plus self-reported racial/ethnic discrimination should be taken in this context. While the exclusion of outliers (those with bronchodilator responses greater than 60%) reduces the standard deviations, similar results to the ones we presented here were observed, suggesting the association between BDR and reports of racial/ethnic discrimination were not influenced or driven by the outliers. Third, because asthma is an inflammatory disease, TNF-α, a marker of inflammation may be elevated in youth with asthma as a result of the underlying disease and altered by controller medications, which have anti-inflammatory properties. Our analyses included controller medication use and a marker of lung function severity as covariates to help address these issues. Fourth, we excluded 433 participants due to missing data mostly for discrimination measures, pulmonary function measures, or TNF-α measurements. However, participants were selected based on disease status and not on reports of racial/ethnic discrimination and/or spirometry measures, and thus, it is unlikely that these exclusions have biased 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 [5,47]. In addition, we previously found that racial/ethnic discrimination, ascertained using these questions, was associated with asthma related outcomes in a pediatric population [3]. Despite these limitations, these data were obtained from a well conducted case control study [3] collecting 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 racial/ethnic 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 racial/ethnic discrimination as a form of 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.

Conclusions

Our study confirms previous findings that psychosocial stress impacts asthma outcomes in children [2,3]. We found BDR to be increased in participants who self-reported racial/ethnic discrimination with this increase being greater among African American youth with TNF-α high asthma, an asthma type thought to be resistant to traditional asthma medications. This finding is clinically important, as those who are at risk of poor asthma outcomes and were previously thought to be unresponsive to asthma medications [15] may actually be responsive and benefit from adjunct behavioral and/or environmental interventions. These results support the need to screen for racial/ethnic discriminatory experiences among those with moderate-severe asthma as it may help to reclassify asthma type and identify more precise treatments for high-risk population.

Supporting information

S1 Table. Eligibility criteria for participation for SAGE II asthma cases.
(DOCX)

S2 Table. Selected characteristics[a] of participants according to TNF-α status in SAGE II (2006–2014). *Definition of Abbreviations*: HS = high school, SAGEII = Study of African Americans, Asthma, Genes and Environment. [a]Values are reported as numbers (percentages) unless otherwise specified. [b]Refers to the education level of the participant's mother. [c]Racial/ethnic discrimination score was categorized as None (negative answer to all 4 situations) or Any (affirmative answer to one or more situations). [d]Report of any asthma controller medication use in the 2 weeks prior to recruitment including inhaled corticosteroids, long acting beta agonist, and/or montelukast.
(DOCX)

S1 Fig. Bronchodilator response (%) by level of reported racial/ethnic 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.
(TIF)

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References

1. Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. NCHS Data Brief [Internet]. 2012/05/24. 2012;(94):1–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22617340 PMID: 22617340
2. Yonas MA, Lange NE, Celedon JC. Psychosocial stress and asthma morbidity. Curr Opin Allergy Clin Immunol [Internet]. 2012/01/24. 2012; 12(2):202–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22266773 https://doi.org/10.1097/ACI.0b013e32835090c9 PMID: 22266773
3. Thakur N, Barcelo NE, Borrell LN, Singh S, Eng C, Davis A, et al. Perceived Discrimination Associated with Asthma and Related Outcomes in Minority Youth: The GALA II and SAGE II Studies. Chest [Internet]. 2016 [cited 2017 May 2]; Available from: http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/936160/101016jchest201611027.pdf
4. Williams DR, Mohammed SA. Discrimination and racial disparities in health: evidence and needed research. J Behav Med. 2009; 32(1):20–47. https://doi.org/10.1007/s10865-008-9185-0 PMID: 19030981
5. Pachter LM, Bernstein BA, Szalacha LA, Coll CG. Perceived Racism and Discrimination in Children and Youths: An Exploratory Study. Health Soc Work [Internet]. 2010; 35(1):61–9. Available from: http://search.proquest.com/docview/210557457/abstract/76B972DE64EA4517PQ/1%5Cnhttp://media.proquest.com/media/pq/classic/doc/1969768381/fmt/pi/rep/NONE?hl=&cit%3Aauth=Pachter%2C+Lee+M%3BBernstein%2C+Bruce+A%3BSzalacha%2C+Laura+A%3BColl%2C+Cynthia+Garcí PMID: 20218454
6. Lee A, Mathilda Chiu YH, Rosa MJ, Jara C, Wright RO, Coull BA, et al. Prenatal and postnatal stress and asthma in children: Temporal- and sex-specific associations. J Allergy Clin Immunol. 2016; 138(3):740–747.e3. https://doi.org/10.1016/j.jaci.2016.01.014 PMID: 26953156

7. Lu Y, Ho R, Lim TK, Sen Kuan W, Goh DYT, Mahadevan M, et al. Neuropeptide Y may mediate psychological stress and enhance TH2 inflammatory response in asthma. J Allergy Clin Immunol [Internet]. 2015 [cited 2017 May 2]; 135:1061–1063.e4. Available from: http://www.jacionline.org/article/S0091-6749(14)01578-4/pdf https://doi.org/10.1016/j.jaci.2014.10.036 PMID: 25498790
8. Gauthier M, Ray A, Wenzel SE. Evolving concepts of asthma. Am J Respir Crit Care Med. 2015; 192 (6):660–8. https://doi.org/10.1164/rccm.201504-0763PP PMID: 26161792
9. National Heart, Lung and BI. Guidelines for the Diagnosis and Management of Asthma (EPR-3). 2007.
10. Tantisira KG, Fuhlbrigge AL, Tonascia J, Van Natta M, Zeiger RS, Strunk RC, et al. Bronchodilation and bronchoconstriction: Predictors of future lung function in childhood asthma. J Allergy Clin Immunol. 2006; 117(6):1264–71. https://doi.org/10.1016/j.jaci.2006.01.050 PMID: 16750985
11. Coutinho MT, McQuaid EL, Koinis-Mitchell D. Contextual and cultural risks and their association with family asthma management in urban children. J Child Health Care [Internet]. 2013; 17(2):138–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23455872 https://doi.org/10.1177/1367493512456109 PMID: 23455872
12. Koinis-Mitchell D, McQuaid EL, Seifer R, Kopel SJ, Esteban C, Canino G, et al. Multiple urban and asthma-related risks and their association with asthma morbidity in children. J Pediatr Psychol. 2007; 32(5):582–95. https://doi.org/10.1093/jpepsy/jsl050 PMID: 17218338
13. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med [Internet]. 2012; 18(5):716–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22561835 https://doi.org/10.1038/nm.2678 PMID: 22561835
14. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. J Allergy Clin Immunol. 2001; 108(5):753–8. https://doi.org/10.1067/mai.2001.119411 PMID: 11692100
15. Brown SD, Brown LA, Stephenson S, Dodds JC, Douglas SL, Qu H, et al. Characterization of a high TNF-α phenotype in children with moderate-to-severe asthma [Internet]. Vol. 135, Journal of Allergy and Clinical Immunology. 2015 [cited 2017 May 2]. p. 1651–4. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4461527/pdf/nihms-660308.pdf
16. Wesolowska-Andersen A, Seibold MA. Airway molecular endotypes of asthma: dissecting the heterogeneity. Curr Opin Allergy Clin Immunol [Internet]. 2015; 15(2):163–8. Available from: http://graphics.tx.ovid.com/ovftpdfs/FPDDNCGCECMCFF00/fs046/ovft/live/gv025/00130832/00130832-201504000-00010.pdf https://doi.org/10.1097/ACI.0000000000000148 PMID: 25961390
17. Krieger N, Smith K, Naishadham D, Hartman C, Barbeau EM. Experiences of discrimination: Validity and reliability of a self-report measure for population health research on racism and health. Soc Sci Med. 2005; 61(7):1576–96. https://doi.org/10.1016/j.socscimed.2005.03.006 PMID: 16005789
18. Hosnijeh FS, Krop EJM, Portengen L, Rabkin CS, Linseisen J, Vineis P, et al. Stability and reproducibility of simultaneously detected plasma and serum cytokine levels in asymptomatic subjects. Biomarkers [Internet]. 2010 Mar 22 [cited 2017 May 2]; 15(September 2009):140–8. Available from: http://www.tandfonline.com/doi/full/10.3109/13547500903340570
19. Gurrola-Díaz CM, Sánchez-Enriquez S, Oregon-Romero E, García-López PM, Garzón de la Mora P, Bastidas-Ramírez BE, et al. Establishment of a cut-point value of serum TNF-alpha levels in the metabolic syndrome. J Clin Lab Anal [Internet]. 2009; 23(1):51–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19140212 https://doi.org/10.1002/jcla.20289 PMID: 19140212
20. Silvestri M, Bontempelli M, Giacomelli M, Malerba M, Rossi G a, Di Stefano a, et al. High serum levels of tumour necrosis factor-alpha and interleukin-8 in severe asthma: markers of systemic inflammation? Clin Exp Allergy. 2006; 36(11):1373–81. https://doi.org/10.1111/j.1365-2222.2006.02502.x PMID: 17083347
21. Almqvist C, Worm M, Leynaert B. Impact of gender on asthma in childhood and adolescence: a GA2LEN review. Allergy [Internet]. 2007/09/08. 2008; 63(1):47–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17822448 https://doi.org/10.1111/j.1398-9995.2007.01524.x PMID: 17822448
22. Oh SS, Tcheurekdjian H, Roth L a, Nguyen E a, Sen S, Galanter JM, et al. Effect of secondhand smoke on asthma control among black and Latino children. J Allergy Clin Immunol. 2012 Jun; 129(6d):1478–83.e7.
23. Dubow EF, Boxer P, Huesmann LR. Long-term Effects of Parents' Education on Children's Educational and Occupational Success: Mediation by Family Interactions, Child Aggression, and Teenage Aspirations. Merrill Palmer Q (Wayne State Univ Press) [Internet]. 2009; 55(3):224–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20390050%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2853053
24. Ogden CL, Lamb MM, Carroll MD, Flegal KM. Obesity and socioeconomic status in children and adolescents: United States, 2005–2008 [Internet]. Vol. 127, NCHS data brief. 2010. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21211166

25. Thakur N, Martin M, Castellanos E, Oh SS, Roth LA, Eng C, et al. Socioeconomic status and asthma control in African American youth in SAGE II. J Asthma [Internet]. 2014/03/25. 2014; 51(7):720–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24654704 https://doi.org/10.3109/02770903.2014.905593 PMID: 24654704

26. Kumar R, Seibold MA, Aldrich MC, Williams LK, Reiner AP, Colangelo L, et al. Genetic Ancestry in Lung-Function Predictions. N Engl J Med [Internet]. 2010 [cited 2017 May 2]; 363(4):321–30. Available from: http://www.nejm.org/doi/pdf/10.1056/NEJMoa0907897 https://doi.org/10.1056/NEJMoa0907897 PMID: 20647190

27. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Adv Data [Internet]. 2000; 314(314):1–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11183293

28. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature [Internet]. 2007; 449(7164):851–61. Available from: http://dx.doi.org/10.1038/nature06258 PMID: 17943122

29. Svarstad BL, Chewning BA, Sleath BL, Claesson C. The Brief Medication Questionnaire: a tool for screening patient adherence and barriers to adherence. Patient Educ Couns. 1999; 37(2):113–24. PMID: 14528539

30. Ferris B. Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis. 1978; 118(6 Part 2):1–120.

31. American Thoracic Society. Standardization of Spirometry. Am J Respir Crit Care Med. 1995; 152 (3):1107–36. https://doi.org/10.1164/ajrccm.152.3.7663792 PMID: 7663792

32. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005; 26(5):948–68. https://doi.org/10.1183/09031936.05.00035205 PMID: 16264058

33. R Development Core Team. R: A Language and Environment for Statistical Computing. R Found Stat Comput Vienna Austria [Internet]. 2016;0:{ISBN} 3-900051-07-0. http://www.r-project.org/

34. Brehm JM, Ramratnam SK, Tse SM, Croteau-Chonka DC, Pino-Yanes M, Rosas-Salazar C, et al. Stress and bronchodilator response in children with asthma. Am J Respir Crit Care Med [Internet]. 2015 [cited 2017 May 2]; 192(1):47–56. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4511425/pdf/rccm.201501-0037OC.pdf https://doi.org/10.1164/rccm.201501-0037OC PMID: 25918834

35. McCormick SP, Nezu CM, Nezu AM, Sherman M, Davey A, Collins BN. Coping and social problem solving correlates of asthma control and quality of life. Chron Respir Dis [Internet]. 2014 [cited 2017 May 2]; 11(1):15–21. Available from: http://journals.sagepub.com/doi/pdf/10.1177/1479972313516878 https://doi.org/10.1177/1479972313516878 PMID: 24431407

36. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. Vol. 372, The Lancet. 2008. p. 1107–19.

37. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. Thorax [Internet]. 2002; 57(7):643–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12096210%5Cnhttp://www.ncbi.nlm.nih.gov/pmc/articles/PMC1746367/pdf/v057p00643.pdf https://doi.org/10.1136/thorax.57.7.643 PMID: 12096210

38. Haczku A, Panettieri R a. Social stress and asthma: the role of corticosteroid insensitivity. J Allergy Clin Immunol. 2010 Mar; 125(3):550–8. https://doi.org/10.1016/j.jaci.2009.11.005 PMID: 20153032

39. Tillie-Leblond I, Pugin J, Marquette CH, Lamblin C, Saulnier F, Brichet A, et al. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. Am J Respir Crit Care Med [Internet]. 1999 Feb [cited 2017 May 2]; 159(2):487–94. Available from: http://www.atsjournals.org/doi/abs/10.1164/ajrccm.159.2.9805115 https://doi.org/10.1164/ajrccm.159.2.9805115 PMID: 9927362

40. Brody GH, Yu T, Miller GE, Chen E. Discrimination, Racial Identity, and Cytokine Levels Among African American Adolescents. [cited 2017 May 2]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4409660/pdf/nihms668456.pdf

41. Bailey MT, Kierstein S, Sharma S, Spaits M, Kinsey SG, Tliba O, et al. Social stress enhances allergen-induced airway inflammation in mice and inhibits corticosteroid responsiveness of cytokine production. J Immunol. 2009 Jun; 182(12):7888–96. https://doi.org/10.4049/jimmunol.0800891 PMID: 19494313

42. Williams DR, Collins C. Racial residential segregation: a fundamental cause of racial disparities in health. Public Health Rep [Internet]. 2001 [cited 2017 May 2]; 116(5):404–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12042604 https://doi.org/10.1093/phr/116.5.404 PMID: 12042604

43. Williams DR, Mohammed S a, Leavell J, Collins C. Race, socioeconomic status, and health: complexities, ongoing challenges, and research opportunities. Ann N Y Acad Sci. 2010 Feb; 1186:69–101. https://doi.org/10.1111/j.1749-6632.2009.05339.x PMID: 20201869

44. Walker TJ, Reznik M. In-school asthma management and physical activity: children's perspectives. J Asthma. 2014 Oct; 51(8):808–13. https://doi.org/10.3109/02770903.2014.920875 PMID: 24796650

45. van Gent R, van Essen-Zandvliet EEM, Klijn P, Brackel HJL, Kimpen JLL, van Der Ent CK. Participation in daily life of children with asthma. J Asthma. 2008 Nov; 45(9):807–13. https://doi.org/10.1080/02770900802311477 PMID: 18972300

46. Kerstjens HA, Brand PL, Quanjer PH, van der Bruggen-Bogaarts BA, Koëter GH, Postma DS. Variability of bronchodilator response and effects of inhaled corticosteroid treatment in obstructive airways disease. Dutch CNSLD Study Group. Thorax [Internet]. 1993 [cited 2017 May 3]; 48(7):722–9. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC464656/pdf/thorax00379-0044.pdf PMID: 8153921

47. Priest N, Paradies Y, Trenerry B, Truong M, Karlsen S, Kelly Y. A systematic review of studies examining the relationship between reported racism and health and wellbeing for children and young people. Soc Sci Med [Internet]. 2013; 95:115–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23312306 https://doi.org/10.1016/j.socscimed.2012.11.031 PMID: 23312306