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Katherine J. Baxter Date

Soluble IL-6 receptor in pediatric severe asthma: relationship to asthma control and interaction with race

By
Katherine J. Baxter, B.S.
Master of Science
Clinical Research

_____
Anne M. Fitzpatrick, Ph.D.
Advisor

_____
Mitchell Klein, Ph.D.
Committee Member

_____
Beau Bruce, M.D.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____
Date

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Katherine J. Baxter
B.S., University of Georgia, 2007

Advisor: Anne M. Fitzpatrick, PhD

An abstract of
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ABSTRACT

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by Katherine J. Baxter

Introduction: Asthma affects up to 10% of all children in the U.S. A subset of these children has severe or refractory asthma, characterized by continuing symptoms despite maximal therapy with corticosteroids. Increased inflammation has been shown molecularly in children with severe asthma, including as one of many markers, increased IL-6. A recent development is the discovery of the soluble IL-6 receptor (sIL-6R) which, unlike the membrane-bound receptor, is found throughout the body and may be responsible for the action of IL-6 in the lung. We hypothesized that plasma IL-6 and sIL-6R expression would be increased in children with severe asthma vs. mild-to-moderate asthma. We then formed a data-driven hypothesis that sIL-6R expression is related to asthma control (independent of treatment regimen) and that the effect of sIL-6R on asthma control is modified by race.
Methods: Banked plasma and extensive characterization data was available for 129 children with asthma enrolled in a previous cohort study. Protein expression of IL-6 was measured using a microsphere-based kit and sIL-6R was measured by ELISA.
Results: We found no difference in IL-6 or sIL-6R expression between severe and mild-to-moderate asthmatics classified by the American Thoracic Society (ATS) definition. However, in a logistic model of very poorly controlled (VPC) asthma classified by the NHLBI asthma control definition, sIL-6R expression was a significant predictor among Caucasian children (OR 3.34 for a 10ng/mL increase, 95% CI 1.18-9.43), but not in children of other races (OR 1.33, 95% CI 0.73-2.44).
Conclusions: These findings are supported by genetic studies of the asthma susceptibility SNP in the coding region of sIL-6R which has been found in populations of European ancestry. This complex relationship provides a starting point for future research into the genetic basis of asthma susceptibility and poor asthma control with conventional therapies. Future research may eventually lead to novel treatments for corticosteroid-resistant patients.

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INTRODUCTION

Asthma is a highly prevalent condition both in adult and pediatric populations, affecting up to 10% of all children (1). Asthma is characterized by inflammation of the airways and reversible bronchoconstriction. The importance of inflammation in the pathophysiology of asthma is demonstrated by the effectiveness of corticosteroid treatment in most patients. However, in some patients with severe asthma, even high dose inhaled or systemic corticosteroids do not provide adequate symptom control. This relatively small group of severe patients accounts for a large proportion of asthma-related morbidity, mortality, and healthcare costs (2). Until recently, the clinical characteristics of pediatric severe asthma were not well delineated. Fitzpatrick et al. have shown that children with severe asthma have reduced lung function (i.e., FEV_1 and FEV_1/FVC) representing airway obstruction, air trapping, increased sensitivity to methacholine bronchoprovocation, greater allergic sensitization by serum IgE and skin prick testing, increased airway inflammation as measured by increased exhaled nitric oxide (F_{ENO}) concentrations, and an increased frequency of exacerbations (3).

The molecular basis for chronic, corticosteroid-resistant inflammation in severe asthma patients is still poorly understood. Measurement of cytokines and chemokines in the bronchoalveolar lavage (BAL) of children with asthma

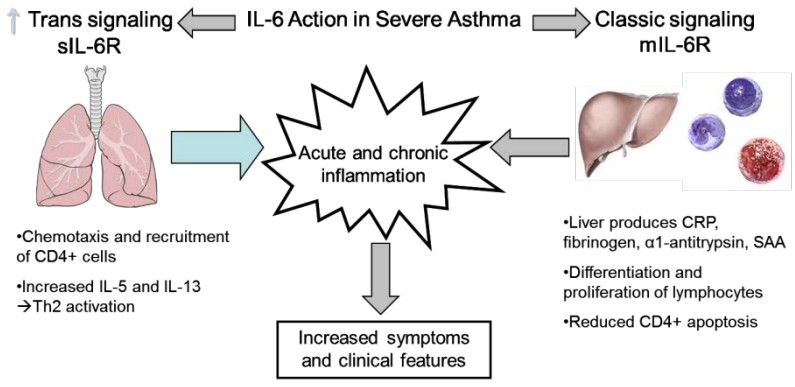

Figure 1 Hypothesized mechanisms and contributions of the soluble IL-6 signaling pathway in severe asthma

showed that IL-6 was increased in asthma versus control subjects. In alveolar macrophage lysate, IL-6 concentrations further differentiated children with severe asthma from moderate asthmatics (4). Thus IL-6 is likely an important inflammatory mediator in asthma and an important marker of the severe asthma phenotype (Figure 1).

There have been recent advances in the understanding of IL-6 signaling which are relevant to the pathogenesis of asthma. The membrane bound IL-6 receptor (mIL-6R) is expressed only on hepatocytes and certain leukocytes. However, a soluble form of the IL-6 receptor (sIL-6R) is produced by cleavage of mIL-6R and by alternate mRNA splicing (5, 6). Many soluble receptors act as antagonists by sequestering ligand, but sIL-6R is an agonist. The sIL-6R binds its ligand IL-6 and then binds to membrane protein gp130 which is ubiquitously expressed, explaining IL-6 action on other cell types. Therefore sIL-6R may be responsible for the action of IL-6 in the lung.

A recent study in asthmatic adults found increased sIL-6R in the BAL of patients with asthma versus control subjects, which increased further with allergen challenge. Concentration of sIL-6R correlated with the number of CD4 cells in the BAL and with the T_h2 cytokines IL-5 and IL-13, suggesting that sIL-6R contributes to T_h2 immune activation in asthma, thereby contributing to chronic inflammation (7). The expression pattern and actions of IL-6 and sIL-6R in pediatric severe asthma is unknown. **We tested the hypothesis that expression of both IL-6 and sIL-6R is increased in pediatric severe asthma and is related to poor symptom control.**

BACKGROUND

Over decades of research, scientists have worked toward understanding in detail the inflammatory milieu of the asthmatic airway. Many important cellular and molecular changes are found in both the airway and plasma of asthma patients. The number of cytokines identified as important players in asthma is large and includes TNFα, TGFβ, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, and IL-8 to name a few (8). Interleukin-6 (IL-6) has consistently been found to be involved in numerous aspects of the pathogenesis of asthma, including the establishment of atopy and chronic inflammation which are essential to the development of this disease.

The IL-6 protein was first cloned by Hirano and colleagues in 1986 (9). It is an important inflammatory mediator in a vast number of conditions including sepsis, injury, infection, and autoimmune disease (10-12). The physiologic functions of IL-6 are diverse. It was first identified as a factor in humoral immunity, inducing differentiation of B-cells into immunoglobulin secreting plasma cells (13). It is a hematopoietic stimulant of erythrocytes, monocytes, granulocytes and platelets. IL-6 is a key player in the acute inflammatory response and stimulates the liver to produce acute phase proteins such as C-reactive protein and serum amyloid A (14, 15). The primary cellular sources of IL-6 secretion are macrophages, monocytes and T-cells. The release of IL-6 is stimulated by lipopolysaccharide (LPS), TNFα, and IL-1 among others (16, 17). Thus IL-6 is a key player in the complex molecular cascade that drives inflammation.

IL-6 expression is increased in asthma

The plasma concentration of IL-6 along with other proinflammatory and Th2 cytokines is increased in asthmatic patients versus healthy controls (18). Early work

showed an increase in IL-6 in the bronchoalveolar lavage fluid (BAL) of asthma patients versus control subjects (19), as well as in symptomatic versus asymptomatic asthma patients (8). Immunohistochemistry suggested that the increased IL-6 is produced by local airway epithelial and monocytic cells as opposed to diffusion alone. In addition, IL-6 was found to be increased in the sputum of asthmatics versus controls (20). IL-6 concentration increased significantly from baseline in the sputum of mild asthmatics experimentally infected with rhinovirus, and in the BAL of asthmatics exposed to common allergens (21, 22). This body of evidence supports the view of a chronic inflammatory state in asthma, exacerbated by acute infection or allergen challenge. A recent study showed an inverse correlation between IL-6 concentration in sputum and FEV_1, supporting the clinical relevance of IL-6 levels to pulmonary function (23).

Functions of IL-6 in asthma

At a fundamental level, IL-6 is important to the overall allergic phenotype in asthma. The upregulation of IL-6 is directly related to the overactive Th2 response in asthma, leading to increased eosinophilia and IgE expression. IL-6 contributes both to the promotion of Th2 differentiation of T cells, and the suppression of the Th1 pathway (24). In addition to differentiation, IL-6 appears to recruit CD4+ T-cells to the airway (7). Mast cells localized to airway smooth muscle bundles is a defining pathologic feature of asthma. In vitro experiments have shown that airway smooth muscle cells produce IL-6, which acts as an essential survival and proliferation factor for mast cells (25). IL-6 is also implicated in airway fibrosis and remodeling in asthma (26). Gomes and colleagues found that fibroblasts in coculture with eosinophils or eosinophil-conditioned media had increased IL-6 expression and increased extracellular matrix-

associated gene expression (27). IL-6 is also expressed by and stored in the granules of eosinophils from asthmatic patients. The release of IL-6 by eosinophils is stimulated by the Th1-type cytokine IFNγ, suggesting an interface between Th1 and Th2-type responses (28). Thus IL-6 is a player in many complex cell-cell interactions which contribute to the hyperresponsive and profibrotic phenotype of the asthmatic airway.

Trans signaling may mediate IL-6 action in the lung

IL-6 can act on cells through a membrane bound receptor (IL-6R also known as the α chain) in what is termed classical signaling. Once IL-6 is bound to IL-6R, this complex recruits a homodimer of the ubiquitously expressed transducer protein gp130 (or β chain). The cellular signaling cascade is then activated which involves the JAK family of tyrosine kinases and the STAT3 transcription factor pathway (29). However, membrane bound IL-6R (mIL-6R) is found only in the liver and on certain hematopoetic cells (30). In contrast, soluble IL-6R (sIL-6R) can bind IL-6 and act on membrane bound gp130 which is expressed by most cells. While other soluble receptors act as antagonists by sequestering ligand, the sIL-6R and IL-6 complex is an agonist. The sIL-6R is generated both by cleavage of membrane bound IL-6R, and by an alternatively spliced mRNA transcript which does not contain the membrane-spanning region (5, 31). This trans signaling pathway helps to explain the actions of IL-6 on diverse cell types, and has been of increasing interest in many disease processes, including asthma, arthritis, inflammatory bowel disease, and cancer.

Soluble IL-6 receptor signaling may be increased in asthma

In a study of a newly identified asthma susceptibility locus Ala^{358}, which is found in subjects of European ancestry, sIL-6R expression was increased in subjects with the

single nucleotide polymorphism (SNP). This finding suggests that there may be a racial difference in the expression of sIL-6R or its relationship with asthma severity. In addition sIL-6R levels correlated with decreased FEV_1 and FVC (32). Dognanci and colleagues found significantly increased sIL-6R in the BAL of asthmatic adults compared to healthy controls, and that sIL-6R expression further increased with allergen challenge in these patients (7). The authors found that sIL-6R was positively correlated with the number of CD4+ cells and eosinophils in the BAL after allergen challenge, as well as with the concentration of Th2 cytokines IL-5 and IL-13, suggesting that trans signaling is important in promoting Th2 activation and atopy in asthma. They blocked sIL-6R specifically using a chimeric gp130Fc protein in a murine model of asthma, and showed decreased levels of Th2 cytokines and the master transcription factor for Th2 cytokines, GATA3, as well as decreased eosinophils in the airway. There was an increase of CD4+CD25+ T-regulatory cells with blockade of both mIL-6R and sIL-6R using anti-IL-6R antibody, but not with sIL-6R blockade alone, suggesting unique roles of the two signaling pathways. Additionally, CD4+CD25+ cells from anti-IL-6R treated mice produced more IL-10 than cells from untreated mice, but a similar effect was not observed in mice with sIL-6R blockade only. Thus IL-6 has important effects on Treg proliferation and immunosuppressive action, but these differ between classical and trans signaling pathways. The potential unique functions of trans signaling in asthma and other diseases remain to be discovered.

IL-6 in pediatric asthma

IL-6 has been shown to be increased in the plasma of children with asthma versus healthy controls (33). As in adults, the increased expression of this cytokine is thought to

relate to activation of the Th2 and eosinophilic immune pathway, which contributes to chronic inflammation in these patients. Measurement of cytokines and chemokines in the BAL of children with asthma showed that IL-6 was increased in asthma versus controls. Additionally, IL-6 concentration in the alveolar macrophage lysate differentiated severe from mild-to-moderate asthma in linear discriminate analysis (4). To date, no studies have examined sIL-6R in pediatric asthma.

HYPOTHESIS AND AIMS

The underlying inflammatory and molecular factors leading to the development of severe asthma and steroid treatment refractoriness in pediatric patients remain unclear. In fact, until recent work by the mentor of this project, even the clinical characteristics of pediatric severe asthma were not well delineated (3). There is a lack of treatment options for those already receiving maximum corticosteroid therapy. The recent characterization of sIL-6R in adult asthmatics presents a novel candidate for biomarker and/or treatment research if its expression is related to asthma severity in children. For this reason we sought to measure IL-6 and sIL-6R expression in children with asthma.

We hypothesized that expression of the cytokine IL-6 and its soluble receptor, sIL-6R, are increased in children with severe asthma as compared to children with mild-to-moderate asthma and that these levels are associated with poor symptom control. We also formed a data-driven hypothesis (supported by the above mentioned recent literature concerning the genetic link of sIL-6R and asthma in people of European descent (32)) that race was a strong effect modifier of the relationship between sIL-6R and asthma control. Our specific aims were to: (1) determine whether protein expression of IL-6 and sIL-6R are increased in the systemic circulation of children with severe vs. mild-to-moderate asthma, (2) determine whether protein expression of sIL-6R predicts poor asthma control (defined by National Heart, Lung and Blood Institute (NHLBI) criteria) as opposed to good-to-moderate control, (3) determine the relationship between race, sIL-6R expression and asthma control.

METHODS

Subjects

Cytokine and receptor expression was measured in banked plasma samples from children with asthma, collected during an ongoing IRB approved cohort study of pediatric asthma at Emory Children's Center (n=140) by the Fitzpatrick laboratory. For this project, only baseline samples and patient characteristics were used, resulting in a cross-sectional design. Inclusion criteria for the cohort study are children ages 5-17 who met all criteria for persistent asthma, and had at least 12% reversibility of FEV_1 with bronchodilator treatment (4). Children with immunodeficiency, recent pneumonia, chronic aspiration syndromes, birth before 35 weeks of gestation, or acute infections were excluded. Because this study used frozen, banked plasma and some children in the study refused blood draws, 13 subjects did not have plasma available for analysis and were excluded from the protein expression analysis.

Severe asthma was diagnosed according to criteria developed by the Severe Asthma Research Program (34) and based on the American Thoracic Society (ATS) guidelines (35)(Table A). Subjects were also classified by the National Heart, Lung, and Blood Institute/ National Asthma Education and Prevention Program (NHLBI/NAEPP) guidelines asthma control scale (36)(Table B). The definition of high-dose corticosteroids was >440 mg fluticasone equivalent per day for children younger than 12 and >880 mg for children 12 to 17 years of age, and consistent use of corticosteroids was confirmed through pharmacy records. Informed consent and assent were obtained from all participating children.

Subject characterization

Children previously underwent comprehensive phenotypic characterization consisting of questionnaires and serum immunoglobulin E and eosinophil quantification. Spirometry was performed with a portable spirometer (KoKo® PDS, Ferraris, Louisville, CO) from the best of three forced vital capacity maneuvers and was interpreted according to population reference equations. Whole blood (25 mL) was collected into heparinized tubes containing a density gradient (Vacutainer® CPT™, Becton, Dickinson and Company, Franklin Lakes, NJ). Subjects were administered general health assessment questionnaires and the Asthma Control Questionnaire (ACQ) upon which asthma control classification was based (37).

Sample preparation

Blood samples were centrifuged at 1800 RCF for 20 minutes at 25°C to separate the peripheral blood mononuclear cells (PBMCs). Plasma supernatant was frozen at 80°C in 250μL aliquots with protease inhibitor (4).

Protein expression

IL-6 protein expression was measured in the plasma of children with asthma using microsphere-based multiplex kits (HSCYTO-60SK Millipore, Billerica, MA). This assay has a maximum sensitivity of 0.10 pg/mL for IL-6. sIL-6R protein expression was measured using a commercial ELISA kit (DR600 Quantikine, R&D Systems, Minneapolis, MN). Soluble gp130, a naturally occurring inhibitor of sIL-6R, was also measured in plasma using an ELISA kit (DGP00 Quantikine). Banked plasma was

thawed at room temperature and diluted 100X using the proprietary calibrator diluent prior to assay use.

Statistical analysis

Data analysis was performed with SPSS software (Version 20). Data were graphically displayed and checked for distribution and outliers before analysis. Differences in IL-6 and sIL-6R protein expression between children with severe and mild-to-moderate asthma were assessed with a two-sample independent t-test for normally distributed variables or the Mann-Whitney U test for non-normally distributed variables. The chi square test of independence was used to compare categorical variables. Significance was defined as $\alpha < 0.05$ using two-tailed tests of significance. Additional univariate analysis using ANOVA was conducted to compare protein expression between the three NHLBI asthma control groups. Prior to multivariate analysis, asthma control groups were stratified by various covariates to examine for interaction. Potential confounders were examined by comparing protein expression between groups and association with asthma severity. Covariates were selected based on known associations with asthma severity and significant associations in our data. Linear regression was performed with plasma sIL-6R concentration as the dependent variable and either asthma severity or asthma control group as the independent variable of interest, controlling for gender, race, body mass index (BMI), and inhaled corticosteroid (ICS) dose. Similarly, logistic regression was performed with probability of being in the very poorly controlled (VPC) asthma control group as the outcome variable, with sIL-6R concentration as the predictor of interest and controlling for the covariates above.

RESULTS

ATS group analysis

Banked plasma was available for 56 mild-to-moderate and 71 severe subjects with asthma. Baseline characteristics of all subjects are shown in Table 1. Plasma IL-6 was non-normally distributed and using nonparametric testing there was no significant difference between the nonsevere and severe groups as defined by ATS guidelines (median 8.47 pg/mL vs. 1.48 pg/mL, $p = 0.112$) (Figure 1). Expression of sIL-6R did not differ between the groups (34.51 ± 10.95 ng/mL vs. 31.32 ± 11.18 ng/mL, $p=0.109$) (Figure 2). Soluble gp130 expression was also similar between ATS nonsevere and severe groups (210.02 ± 59.11 ng/mL vs. 197.07 ± 59.19, $p=0.221$) (Figure 3). Univariate analysis was carried out for potential confounders, comparing the mean sIL-6R concentration between groups (Table 2). Expression of sIL-6R was significantly higher in Caucasians vs. non-Caucasians (41.1 ± 12.1 vs. 30.4 ± 9.7, $p<0.0001$).

The covariates gender, race (white vs. nonwhite), and BMI (normal, overweight, or obese) were selected based on known associations with asthma severity and on our findings in Table 1 and Table 2. Multivariate analysis controlling for gender, race, and BMI found that plasma IL-6 expression was not a significant predictor of severe asthma (OR 0.96 per 10pg/mL increase, 95% CI 0.90-1.03), nor was sIL-6R expression (OR 0.96 per 10ng/mL increase, 95% CI 0.66-1.40). Confounding by ICS treatment could not be adequately controlled in this analysis, because ICS dose is one of the key factors in ATS classification (Table A).

NHLBI group analysis

NHLBI guideline classification does not consider ICS dose, but only symptom burden and lung function (Table A). Features of the subjects are shown in Table 1. In univariate analysis, plasma IL-6 did not differ between well controlled vs. not well controlled vs. very poorly controlled (VPC) groups (median 0.04 vs. 7.85 vs. 4.72 pg/mL, p= 0.099), nor did plasma sIL-6R expression (Figure 4). When groups were stratified by race, potential effect modification was observed (Figure 5).

In multivariate analysis, the very poorly controlled (VPC) group was chosen as the group of interest and compared to a composite of the not well controlled and well controlled groups. In linear regression modeling sIL-6R expression, VPC asthma (controlling for gender, race, BMI, and ICS dose) predicted a significantly higher sIL-6R level by 4.73 ng/mL (95% CI, 0.30-9.15). Analogously in logistic regression modeling the probability of being in the VPC group (controlling for the same covariates), sIL-6R expression was a significant predictor with an odds ratio (OR) 1.76 (95% CI, 1.05-2.95) for a 10ng/mL increase in sIL-6R. Including an interaction term for sIL-6R by race, race specific ORs were calculated (Table 4). A significant effect of sIL-6R was found in Caucasians but not in patients of other races (OR 3.34 for a 10 ng/mL increase, 95% CI 1.18-9.43). Expression of IL-6 was not a significant predictor of VPC asthma when modeled with the same covariates above (OR 1.05 for a 10 pg/mL increase, 95% CI 0.96-1.16). Among the covariates, overweight (OR 3.39, 95% CI 1.02-11.27) and obesity (OR 6.55, 95% CI 1.74-24.61) were significant predictors of VPC asthma, and high dose ICS was near-significant (OR 4.97, 95% CI 0.94-26.39) (Table 5).

DISCUSSION

We sought to measure plasma IL-6 and sIL-6R expression in the plasma of children with severe and non-severe asthma. The ubiquity throughout the body of the soluble receptor, in contrast to the membrane-bound receptor, makes the former a strong candidate for the mediator of IL-6 action in the lung. Recent work in adults suggests that increased sIL-6R is associated with the manifestation of asthma and with reduced lung function in these patients. We therefore measured plasma sIL-6R and investigated its relationship with both asthma severity and asthma control (independent of therapy) in children.

We found no relationship between plasma IL-6 or sIL-6R and asthma severity as defined by the ATS guidelines. One possible explanation for this unexpected finding lies in the definition of asthma severity. The major required criterion for a diagnosis of severe asthma is the use of high-dose inhaled corticosteroids (ICS) or continuous oral corticosteroids (35). As a powerful and broad-acting anti-inflammatory agent, corticosteroids are known to reduce the production of inflammatory cells and the expression of cytokines and pro-inflammatory receptors (38) in responsive patients. Although the response of sIL-6R expression to corticosteroid treatment has not been specifically investigated, it is a reasonable supposition that inhaled and/or systemic corticosteroids may suppress sIL-6R. That idea is supported by our data, in which we noted a negative correlation between ICS dose and sIL-6R expression, and may explain why the severe asthma group (which is composed only of patients treated with high dose corticosteroids) did not have increased sIL-6R and in fact trended toward lower expression levels compared to the mild-to-moderate group. Another issue with the ATS

definition is that it does not clearly delineate patients responsive to corticosteroids from those who are refractory. Because the ATS asthma severity definition includes corticosteroid treatment, it was not possible to control for ICS in order to tease out the effect of sIL-6R independent of treatment. This challenge led us to reconsider our outcome variable classification, and we arrived at the symptom-based NHLBI asthma control classification. In addition to corticosteroid use, we also examined other potential confounders such as gender, race, and BMI.

Among children, asthma is more prevalent in males and tends to develop earlier in life (1). For unknown but possibly hormonal reasons, this trend reverses in the adult asthma population with higher prevalence among adult women than men. Males with asthma also tend to be more atopic (higher IgE and greater allergic sensitization) than females (39, 40). We found that gender was similarly distributed between ATS severe and nonsevere groups, but that patients with very poorly controlled (VPC) asthma were more likely to be male. Interestingly, there was a near-significant trend toward higher plasma sIL-6R in female subjects vs. male subjects, suggesting a possible difference in inflammatory profile between the sexes. Gender has been associated with asthma control in a Hispanic pediatric asthma cohort (41). In light of the above evidence, we adjusted for gender in our multivariate analysis, despite the fact that it was not a significant predictor in our model.

In our cohort, children with VPC asthma were more likely to be overweight or obese. In multivariate analysis, both overweight and obesity were significant predictors of VPC asthma. Our finding agrees with previous work which has shown that obese patients have inferior asthma control (42), and that this may in part be due to an

attenuated response to corticosteroids (43, 44). Although the mechanism behind the association of BMI and asthma is unclear, obesity is known to be a state of increased systemic inflammation (45, 46). Inflammation in obese people with asthma, however, appears to be a non-eosinophilic and Th1 predominant process (47). Additionally, because symptom indices are somewhat subjective, overweight or obese patients may have a contribution of deconditioning and mechanical airflow restriction which increases symptom burden, but is not necessarily related to the asthma disease process. For these reasons, overweight and obese asthma patients may have biologic differences which influence airway inflammation, and BMI is an important potential confounder in our study.

In the univariate analysis, we noted a strong effect of race on the expression of sIL-6R, specifically that expression was higher overall in Caucasians. Additionally when NHLBI asthma control groups were stratified by race, there appeared to be a clear relationship between sIL-6R expression and asthma control among Caucasians, but no discernible relationship among non-Caucasians. This finding is especially interesting in light of the recent identification of a single nucleotide polymorphism (SNP) in the IL-6R coding region as an asthma susceptibility locus (48). This SNP is strongly associated with the plasma concentration of sIL-6R (49). The genome-wide association study (GWAS) was conducted in an Australian cohort of European descent. Our patient population at Emory Healthcare in Atlanta, Georgia on the other hand is diverse, and the pediatric asthma clinic population from which our subjects were drawn is majority African American. The results of our study raise the question of a different genetic basis for asthma susceptibility in patients of African heritage. Asthma is more prevalent

among African American children, even adjusting for socioeconomic status (50) and tends to be more severe (3). Studies in adults of African heritage have shown increased serum IgE, increased incidence of hospitalization for asthma, and increased asthma mortality in this population (1, 51). Because of these disparities, more asthma research directed toward the African American population is needed and will be a future focus of the Fitzpatrick laboratory.

The effect modification of race on the relationship between plasma sIL-6R expression and asthma control bore out in multivariate analysis, showing a greater and statistically significant effect (OR 3.34, 1.18-9.43) among Caucasian subjects, but a smaller and nonsignificant effect among non-Caucasians (OR 1.33, 0.73-2.44). Although asthma is known to be an allergic, inflammatory process, the specific molecular drivers may vary by population. Recognizing that the concept of race is ill-defined and subjective, and serves as a very rough approximation of genetic background, our results emphasize the growing role of rigorous genetics in asthma research. The potential for routine clinical genetic sequencing and personalized medicine continues to move closer to reality in many fields, including asthma care. For example, genetic sequencing may eventually allow clinicians to identify the most effective anti-inflammatory monoclonal antibodies to administer, as opposed to the current "shotgun" approach using corticosteroids, which have a broad action with many side effects.

In conclusion, we found the relationship between sIL-6R expression and asthma severity to be more complex than we originally hypothesized. In fact, using the ATS definition of asthma severity which is based on corticosteroid use, we found no association between either plasma IL-6 or sIL-6R and severe asthma. In order to allow

adjustment of the model by ICS dose, we analyzed our data using the NHLBI guideline definition of asthma control which is based solely on symptom burden and lung function independent of medication regimen. In this context we found that increased sIL-6R was a significant predictor of very poorly controlled (VPC) asthma, but only among Caucasian subjects. This result provides a basis for future genetic work in asthma, as well as further justification for more studies directed specifically toward the African American population. Knowledge of the genetic link between asthma susceptibility, sIL-6R expression and reduced asthma control may eventually lead to novel treatments for patients who are refractory to current regimens.

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TABLES AND FIGURES

Table A American Thoracic Society (ATS) definition of refractory or "severe" asthma. Adapted from the Proceedings of the ATS Workshop on Refractory Asthma, 2000

Major criteria (1 required for severe asthma)	1. Treatment with oral corticosteroids continuously or near-continuously (>50% of the year) 2. Treatment with high-dose inhaled corticosteroids
Minor criteria (2 required for severe asthma)	1. Daily use of asthma controller medication other than corticosteroids 2. Daily or near-daily use of short-acting β-agonist for symptoms 3. Persistent airway obstruction by lung function testing (i.e. FEV_1 <80% predicted) 4. One or more urgent care visits for asthma in one year 5. Three or more oral steroid "bursts" per year 6. Prompt deterioration of symptom control with ≤ 25% reduction in corticosteroid dose 7. Near fatal asthma event in the past

FEV_1 (forced expiratory volume in one second)

Table B- National Heart, Lung, and Blood Institute (NHLBI) guidelines asthma control classification

Criteria	**Well Controlled**	**Not Well Controlled**	**Very Poorly Controlled**
Symptoms	≤2 days/week	>2 days/week	Throughout the day
Nighttime awakenings	≤1x/month	≥2x/month	≥2x/week
Short acting beta-agonist (SABA) use	≤2 days/week	>2 days/week	Several times per day
FEV_1/FVC % predicted	>80%	75-80%	<75%

FEV1/FVC (forced expiratory volume in one second to forced vital capacity ratio)

Table 1- Characteristics of children with asthma (n=140) classified as severe or nonsevere based on the American Thoracic Society (ATS) criteria

Variable	Nonsevere (n=63)	Severe (n=77)	P-value
Male	38 (60.3)	42 (54.5)	0.492
Nonwhite	37 (58.7)	69 (89.6)	<0.0001
Allergen sensitized	33 (60.0)	37 (82.2)	0.016
BMI **Normal** **Overweight** **Obese**	 32 (50.8) 16 (25.4) 15 (23.8)	 40 (52.6) 16 (21.1) 20 (26.3)	 0.823
Second-hand smoke exposure	11 (17.7)	15 (20.0)	0.737
Age at diagnosis (years)	11.6 ± 3.8	12.3 ± 3.9	0.275
Asthma duration (years)	7.9 ± 4.8	10.5 ± 3.5	<0.0001
Hospitalized in the last year	9 (14.5)	38 (49.4)	<0.0001
Intubated ever	7 (11.3)	34 (44.2)	<0.0001
Daily dose ICS (µg)	309 ± 245	850 ± 219	<0.0001
FEV1 % predicted	99.0 ± 15.6	84.8 ± 17.3	<0.0001
FEV1/FVC ratio	94.5 ± 9.1	85.9 ± 11.8	<0.0001

Data reported as means +/- SDs or number (percentage).

BMI (body mass index), ICS (inhaled corticosteroids), FEV_1 (forced expiratory volume in one second), FEV_1/FVC (FEV_1 to forced vital capacity ratio)

Table 2- Mean sIL-6R expression in children with asthma (n=127) compared between groups of potential confounding variables

Variable	Mean sIL-6R ng/mL	p-value
Gender		
Male	31.15 ± 11.72	0.068
Female	34.80 ± 10.50	
Race		
Caucasian	41.06 ± 12.05	<0.0001
Non-Caucasian	30.37 ± 9.71	
Atopic		
Yes	32.48 ± 10.93	0.640
No	33.60 ± 12.06	
Smoke exposure		
Yes	31.70 ± 11.93	0.609
No	33.01 ± 11.12	
BMI		
Normal	33.74 ± 10.87	0.450
Overweight	32.49 ± 11.97	
Obese	30.72 ± 11.14	
ICS		
None	37.23 ± 11.84	0.386
Low dose	33.45 ± 10.52	
High dose	31.89 ± 11.41	

Data reported as means ± SDs; BMI (body mass index); ICS (inhaled corticosteroids)

Table 3- Characteristics of children with asthma (n=140) classified by the NHLBI asthma control guidelines

Variable	Well Controlled (n=19)	Not Well Controlled (n=21)	Very Poorly Controlled (n=90)	P-value
Male	7 (38.9)	15 (51.7)	57 (63.3)	0.122
Nonwhite	9 (50)	15 (51.7)	79 (87.8)	<0.0001
Allergen sensitized	9 (56.2)	13 (59.1)	47 (77)	0.129
BMI				0.016
Normal	12 (66.7)	20 (69.0)	38 (42.2)	
Overweight/Obese	6 (33.3)	9 (31.0)	52 (57.8)	
Second-hand smoke exposure	1 (5.6)	4 (13.8)	21 (23.6)	0.148
Age at diagnosis	11.25 ± 3.18	11.53 ± 2.85	12.29 ± 4.21	0.444
Asthma duration (years)	8.69 ± 4.21	8.13 ± 4.22	9.79 ± 4.30	0.165
Hospitalized in the last year	1 (5.6)	2 (6.9)	42 (46.7)	<0.0001
Intubated ever	5 (27.8)	4 (13.8)	30 (33.3)	0.128
Daily dose ICS	278 ± 218	528 ± 389	696 ± 326	<0.0001
FEV1 % predicted	98 ± 11	101 ± 17	86 ± 18	<0.0001
FEV1/FVC ratio	0.86 ± 0.04	0.84 ± 0.06	0.75 ± 0.10	<0.0001

Data reported as means +/- SDs or number (percentage).

BMI (body mass index), ICS (inhaled corticosteroids), FEV_1 (forced expiratory volume in one second), FEV_1/FVC (FEV_1 to forced vital capacity ratio)

Table 4- Race specific adjusted odds ratios for very poorly controlled (VPC) asthma vs. well or moderately controlled asthma with a 10ng/mL increase in plasma sIL-6R expression (n=127)

	Adjusted OR*	**95% CI**
sIL6-R 10 ng/mL		
Caucasian	3.34	1.18, 9.43
Non-caucasian	1.33	0.73, 2.44

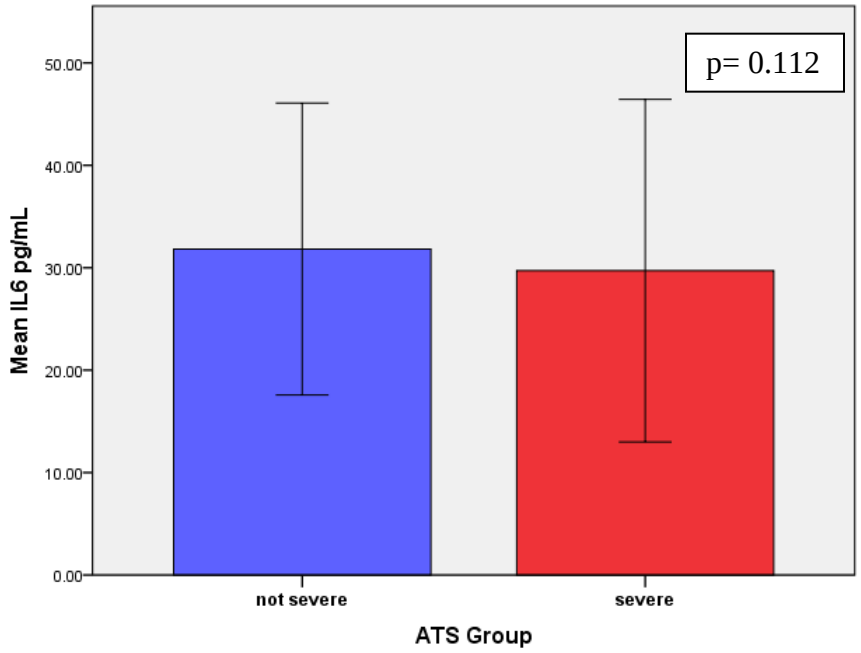
*Adjusted for the effects of gender, ICS (inhaled corticosteroids) dose, and BMI (body mass index)

Table 5- Crude and adjusted odds ratios for very poorly controlled (VPC) asthma vs. well or moderately controlled asthma in children with asthma (n=127)

Variable	**Crude OR**	**Adjusted OR**	**95% CI for adjusted OR**
Gender (male vs. female)	1.96	2.17	0.77, 6.17
NonWhite (nonwhite vs. white)	6.88		
ICS group (low dose vs. none)	0.47	0.54	0.10, 2.97
ICS group (high dose vs. none)	3.89	4.97	0.94, 26.39
BMI group (overweight vs. normal)	2.53	3.39	1.02, 11.27
BMI group (obese vs. normal)	3.37	6.55	1.74, 24.61

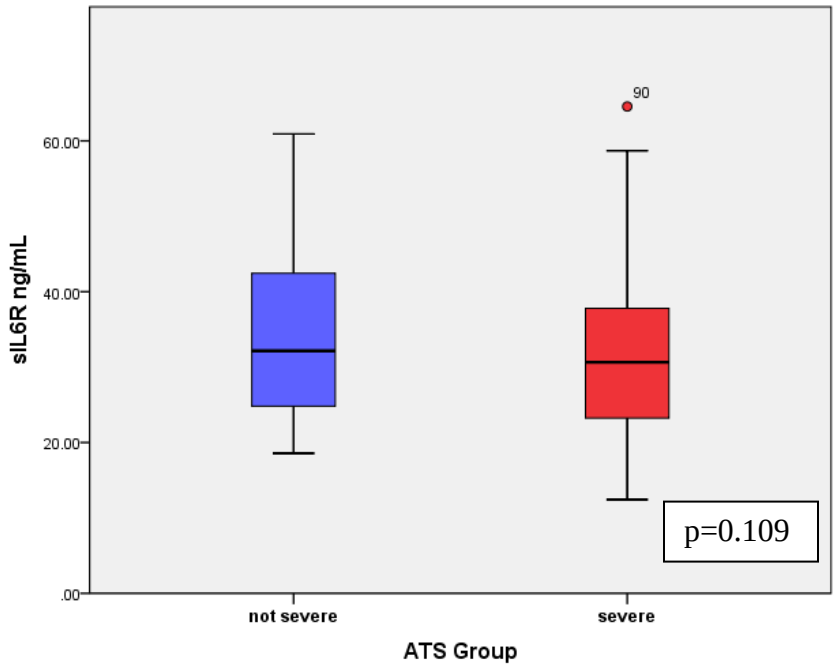
Adjusted odds ratio is calculated from the logistic model containing all of the above covariates in addition to sIL-6R expression, and an interaction term for race and sIL-6R.
ICS (inhaled corticosteroids); BMI (body mass index)

Figure 1- Plasma IL-6 protein concentration pg/mL in severe (n=64) and nonsevere (n=55) pediatric asthma subjects (n=119) bars represent +/- 2 standard errors



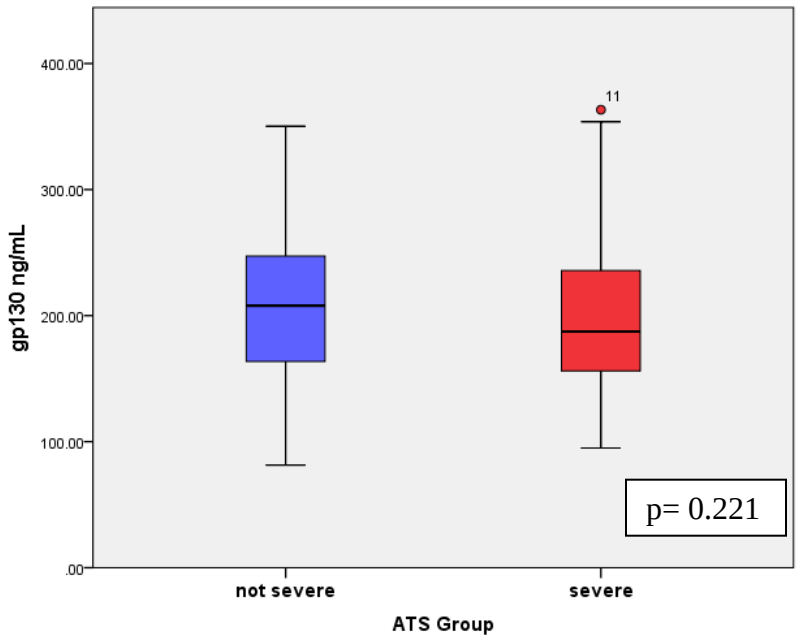
ATS Group (American Thoracic Society definition of asthma severity)

Figure 2- Plasma sIL-6R protein concentration pg/mL in severe (n=71) and nonsevere (n=56) pediatric asthma subjects



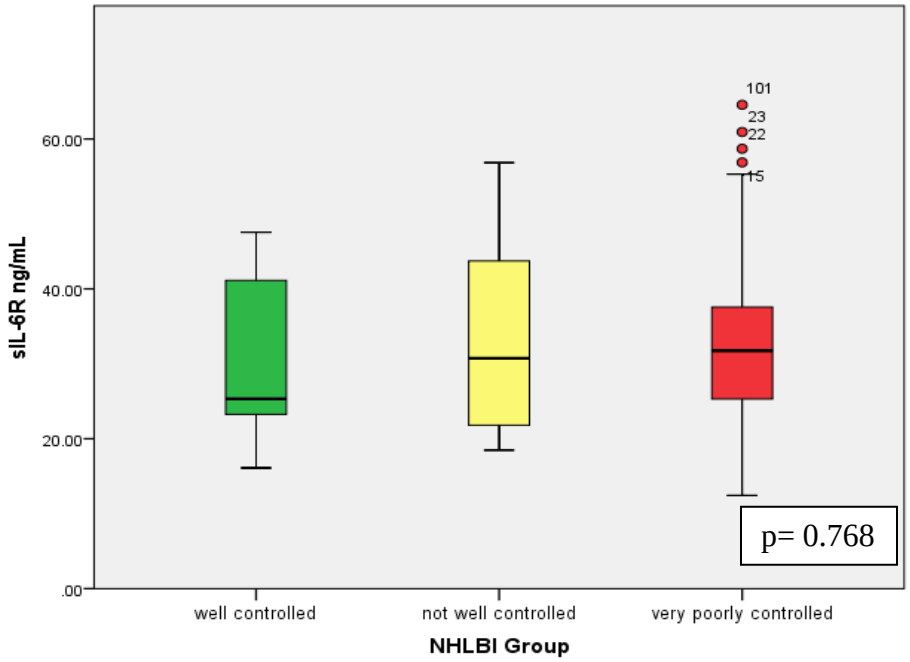
ATS Group (American Thoracic Society definition of asthma severity

Figure 3- Plasma sgp130 concentration ng/mL in children with severe (n=71) and nonsevere (n=57) asthma



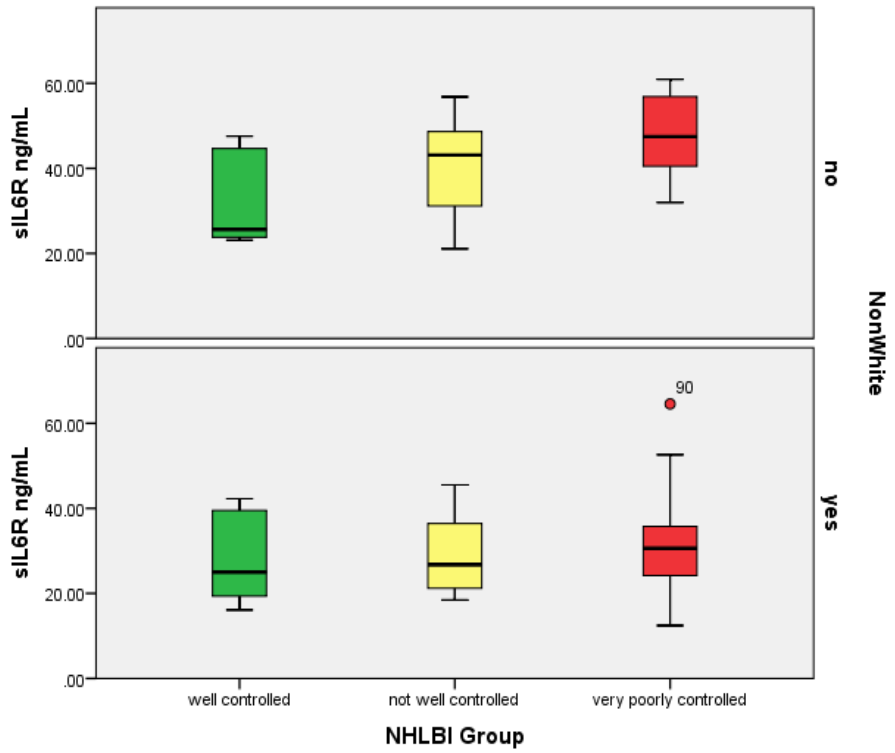
ATS Group (American Thoracic Society definition of asthma severity)

Figure 4- Plasma sIL-6R expression in NHLBI asthma control grouping of children with asthma (n=127)



NHLBI group (National Heart, Lung, and Blood Institute classification of asthma control)

Figure 5- Plasma sIL-6R expression in children with asthma (n=127) in NHLBI asthma control groups, stratified by race



NHLBI group (National Heart, Lung, and Blood Institute classification of asthma control)