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Evidence for an IL-6 high asthma phenotype in asthma patients of African ancestry

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Data analysis and interpretation: SW, BL, CO
Statistical analysis: SW, BL
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Running title: IL-6 high phenotype in African-Americans with asthma

Conflict of Interest

Dr. White is a principal investigator the PrecISE Asthma Network (NHLBI UG1-HL139125) that is considering asthma therapies based on the work cited in reference #5 of the manuscript. Dr. White does not have a direct interest in the outcomes of that consideration. None of the other authors have a conflict of interest with regard to financial support, consultant arrangements, speakers' bureau participation, stock or other equity ownership, patent licensing arrangements,

46 support such as financial or materials grants for research, employment, or expert witness testi-
47 mony.

48 **Capsule summary**

49 High circulating IL-6 may define a phenotype of asthma associated with obesity and systemic
50 inflammation. We demonstrate that circulating IL-6 is higher in African-American patients with
51 asthma, and that race-specific thresholds should be considered.

52 **Key words**

53 Asthma, Interleukin-6, phenotypes, endotypes, biomarker, eosinophils, obesity, race, ethnicity

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To the editor:

Asthma is increasingly recognized as a heterogeneous clinical syndrome with diverse underlying molecular mechanisms. While type 2 (T2) inflammation is important in some patients with asthma, many patients with severe asthma lack T2 airway inflammation, do not respond to current T2-high therapies, and have few therapeutic options (1). Obesity in adulthood co-occurring with asthma has been suggested as a distinct phenotype that is associated with type-1 inflammation, is more common in women, and is more likely to be non-atopic (2, 3). Systemic inflammation related to obesity and low-grade metabolic syndrome, due to production of pro-inflammatory mediators such as interleukin-6 (IL-6) by adipocytes and macrophages, is associated with diabetes, atherosclerosis, and dyslipidemia (4). These circulating mediators may also influence airway inflammation. A recent study of two cohorts of patients with mild to severe asthma suggests that a subset of these patients may belong to a new “IL-6 high phenotype” characterized by high circulating concentrations of IL-6 (5). The “IL-6 high” patients in both cohorts, one a single-center cohort and the other derived from the Severe Asthma Research Program (SARP), had a higher body mass index (BMI) and a higher prevalence of diabetes and hypertension, suggesting an association of this phenotype with metabolic disorders. These patients also had worse lung function and a greater number of asthma exacerbations. Importantly, the cohorts had a racial balance that was majority white (62 and 66%) with relatively few African-Americans (11 and 23%). As African-Americans have a greater prevalence of diseases associated with low-grade systemic inflammation (6, 7), the definition of a “high-IL-6” phenotype may differ based on race.

To examine the role of race on the IL-6 phenotype in asthma, we conducted a prospective, observational clinical study that was approved by the Institutional Review Board at the University of Chicago. A description of the clinical protocol and methods is provided in the *Online Repository*, and data from a subset of these subjects has previously been reported (8, 9). A total of 68 control subjects with no history of pulmonary disease and 124 subjects with asthma defined by

the *EPR 3 Guidelines on Asthma* were studied. The clinical characteristics of the subjects are shown in Table 1. Assays of IL-6 were done using aliquots of ethylenediaminetetraacetic acid-treated plasma and high sensitivity human (2018) IL-6 Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA; lower and upper limit of detection, 0.16 and 10.0 pg/mL, respectively). When higher concentrations were detected, diluted plasma kept at 4°C overnight was assayed again the following day. To calculate a reference value for plasma IL-6 concentrations in control subjects we log-transformed values to normalize the distribution of values (confirmed by kernel density estimate and quantile-quantile plot) and applied the following equation to determine the upper 95th centile value:

$$= \mu_{\log} + t_{(0.95, n-1)} \times \sqrt{(n+1)/n} \times \sigma_{\log}$$

where $t_{(0.95, n-1)}$ is the 95% quantile of a Student's t-distribution with n-1 degrees of freedom, and $\mu_{\log}$ and $\sigma_{\log}$ are the mean and standard deviation of the log distribution of values, respectively. Incorporation of t adjusts for the size of the population. A single-tailed test was used as values defined as below the lower 95th centile threshold were not of clinical interest. The reference threshold value for the entire control population was 4.13 pg/ml; the reference value for subjects of self-reported European ancestry (EA) was 3.69 pg/ml and for subjects of self-reported African ancestry (AA) was 4.89 pg/ml. Eleven subjects (9 control, 2 asthmatic) were of ancestry other than EA or AA; these individuals were not included in the ancestry-specific analyses.

Consistent with the results of the Peters et al. study (5), IL-6 concentrations were higher in patients with asthma (Table 1), and approximately 15% of asthma patients overall had a concentration greater than the total group reference threshold (Figure S1). Patients with asthma and IL-6 concentrations above the threshold had a higher BMI but did not differ with respect to blood eosinophil or neutrophil counts, serum IgE concentration, or FEV1 (Table 2). While the age difference in the high IL-6 group was significant (Table 2), there was no significant relationship between age and plasma IL-6 in patients with asthma ($R^2 = 0.03$, $p = 0.07$). As expected, a higher

BMI correlated with a higher plasma IL-6 concentration in both control and asthmatic subjects (Figure S2). As in the study of Peters, et al. (5), there were no significant correlations between plasma IL-6 concentrations and the number of blood eosinophils, fraction of exhaled nitric oxide (FeNO), or serum IgE in asthmatic subjects (Figure S3).

IL-6 concentrations were higher in AA subjects compared to EA subjects in both control and asthmatic groups (Table 1). IL-6 concentrations were significantly higher in asthmatic subjects of EA ancestry versus EA control subjects, a difference not seen in subjects of AA ancestry. Although the proportion of asthma patients with IL-6 values above the threshold was higher in subjects of EA versus AA ancestry, this difference was not significant (Table 2). However, while 8 of 77 AA asthmatic subjects had IL-6 values above the threshold determined for AA subjects, applying the threshold for EA subjects to the AA subjects would have moved 8 AA subjects from the low to the high IL-6 group, doubling the proportion of AA subjects in the IL-6 high phenotype. Likewise, applying the total group threshold would have moved 6 AA subjects to the IL-6 high phenotype.

We demonstrate that control AA subjects without asthma have higher IL-6 concentrations compared to control EA subjects without asthma, suggesting that a higher threshold value is required in AA subjects to define the IL-6-high phenotype. Using race-specific thresholds, the proportion of patients with the IL-6-high phenotype in each ancestral group with a high IL-6 concentration is appreciable, and concentrations are similar in both groups. We specifically note that a IL-6 concentration above the reference value threshold does not necessarily predict a threshold for drug responsiveness or of clinical significance; such thresholds remain to be determined. Our data suggest that future studies examining the IL-6 high phenotype in asthma should account for racial differences.

Respectfully,

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181 **Table 1. Baseline characteristics of the control and asthmatic subjects in this study.**

Parameter	Control (N = 68)	Asthma (N = 124)
Age, median (IQR)	31 (21, 48)	33.5 (25, 48)
Women, N (%)	43 (63%)	81 (65%)
Ancestry (EA : AA : Other), N	27 : 32 : 9	44 : 77 : 2
BMI, median (IQR)	26.1 (23.5, 31.4)	28.7 (25.1, 34.6)
BMI > 30 kg/m ² , N (%)	19 (28%)	57 (46%) [¶]
History of sinusitis, N (%)	1 (1%)	22 (18%)
History of GERD, N (%)	2 (3%)	22 (18%)
Serum IgE, median (IQR)	39 (17, 157)	134 (39, 383) *
Inhaled corticosteroid use, N (%)	–	72 (58%)
Oral corticosteroid use, N (%)	–	21 (17%)
Blood eosinophils, N / μ L, (mean $\pm$ SD)	123 $\pm$ 82	297 $\pm$ 77 *
Blood eosinophils > 300 / μ L, N (%)	1 (1%)	35 (28%) *
Blood neutrophils, N / μ L, (mean $\pm$ SD)	3718 $\pm$ 1649	3818 $\pm$ 1984
FeNO, ppb, median (IQR)	15 (12, 22)	24 (14, 42) *
FEV1, % predicted (mean $\pm$ SD)	96.2 $\pm$ 12.2	79.2 $\pm$ 21.0 *
FEV1 $\geq$ 80% predicted N (%)	–	65 (52%)
FEV1 60 - 80% predicted (N (%))	–	35 (28%)
FEV1 $\leq$ 60% predicted N (%)	–	24 (19%)

Plasma IL-6 in all subjects, pg/ml (mean $\pm$ SD)	1.77 $\pm$ 1.26	2.69 $\pm$ 3.19 †
Plasma IL-6 in EA subjects, pg/ml (mean $\pm$ SD)	1.58 $\pm$ 1.26	2.67 $\pm$ 4.20 †
Plasma IL-6 in AA subjects, pg/ml (mean $\pm$ SD)	2.15 $\pm$ 1.30 §	2.75 $\pm$ 2.52

¶, P < 0.05 for asthma versus control subjects by Fisher's exact test; *, P < 0.05 for asthma versus control subjects by Mann-Whitney rank sum test; †, P < 0.05 for asthma versus control subjects by Student t test; §, P < 0.05 for EA versus AA subjects by Student t test. Corrections made for multiple tests as required. Abbreviations as in text.

188 **Table 2. Characteristics of patients with asthma in the low and high IL-6 groups** [†]

Parameter (asthmatic subjects <u>only</u>)	Low IL-6 group (N = 105)	High IL-6 group (N = 16)
Age, median (IQR)	32 (24,47)	44 (34.8, 55.8) *
Women, N (%)	64 (61%)	15 (94%) [¶]
Ancestry (EA : AA : Other), N	36 : 69 : 0	8 : 8 : 0
AA subjects in phenotype, N (%)	69 (90%)	8 (10%)
EA subjects in phenotype, N (%)	36 (82%)	8 (18%)
BMI, median (IQR)	28.1 (24.9, 34.0)	42.7 (27.5, 50.3) *
BMI > 30 kg/m ² , N (%)	46 (44%)	11 (69%)
Inhaled corticosteroid use, N (%)	59 (56%)	12 (75%)
Oral corticosteroid use, N (%)	16 (15%)	5 (31%)
Blood eosinophils, N / μ L, median (IQR)	200 (110, 320)	155 (82, 290)
Blood neutrophils, N / μ L, median (IQR)	3200 (2490, 4540)	3690 (2898, 4920)
FeNO, ppb, median (IQR)	25 (14, 44)	16 (7, 33)
Serum IgE, median (IQR)	141 (41, 505)	101 (25, 231)
FEV1, % predicted (mean $\pm$ SD)	79.4 $\pm$ 21.9	75.1 $\pm$ 15.7

189 [†] Race-specific thresholds as defined in the text are used to group EA and AA subjects into the
 190 low and high IL-6 groups.

191 [¶], P < 0.05 for asthma versus control subjects by Fisher's exact test; *, P < 0.05 for asthma ver-
 192 sus control subjects by Mann-Whitney rank sum test. Abbreviations as in text.

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Figure S1

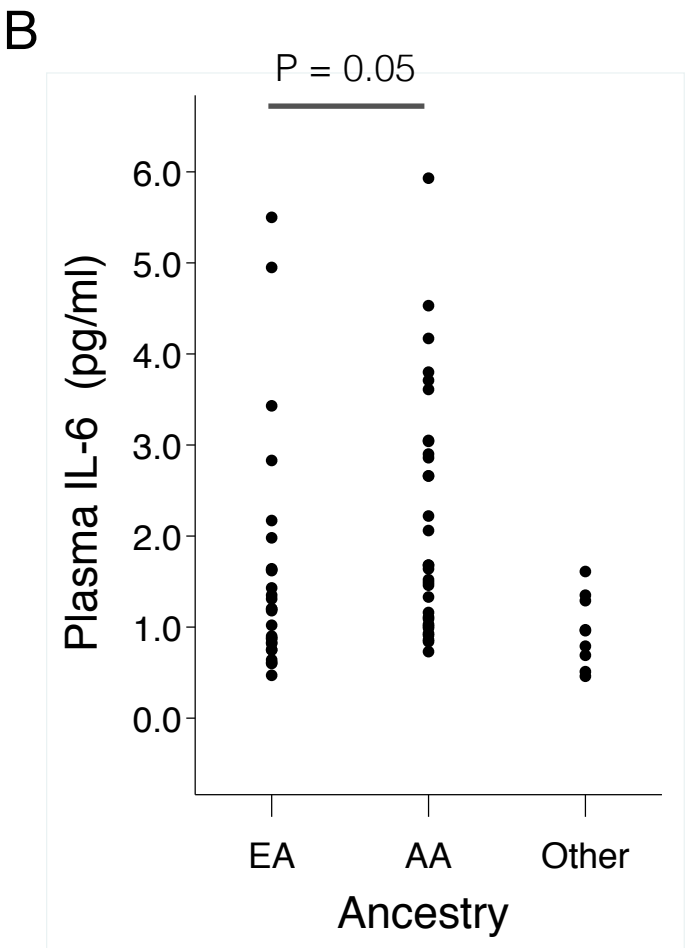
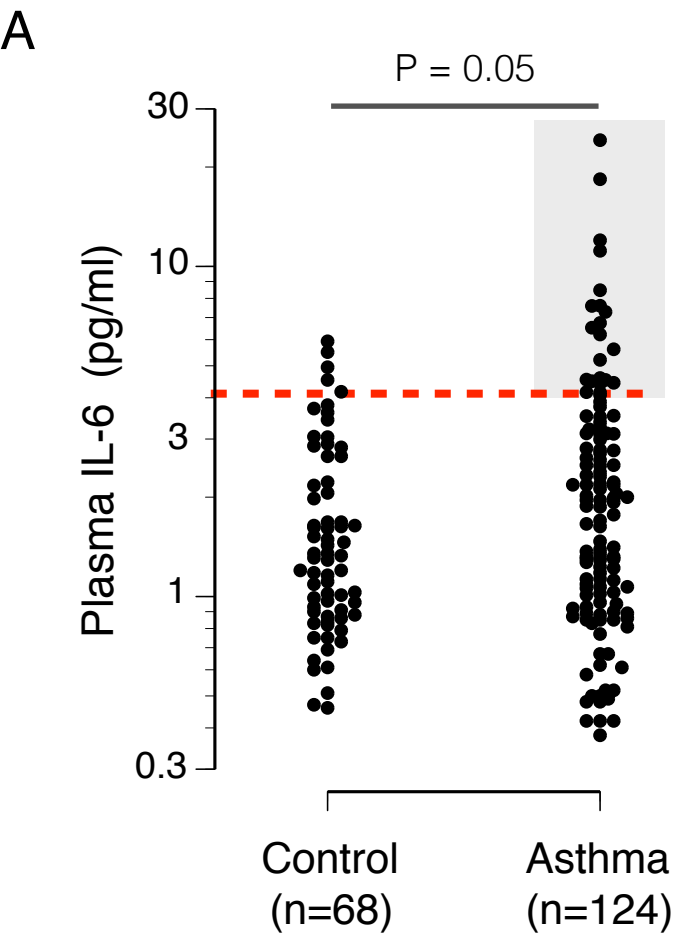


Figure S2

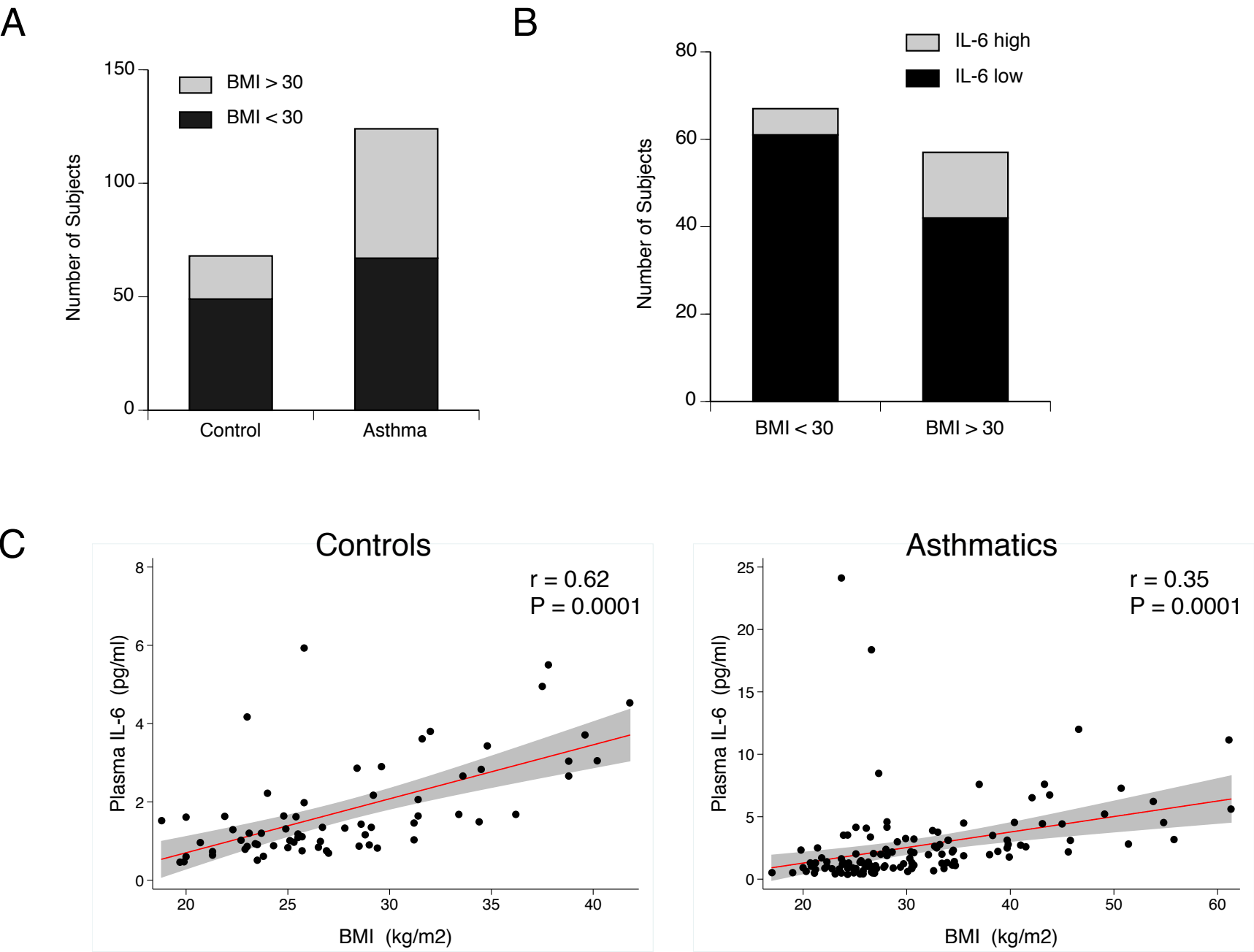
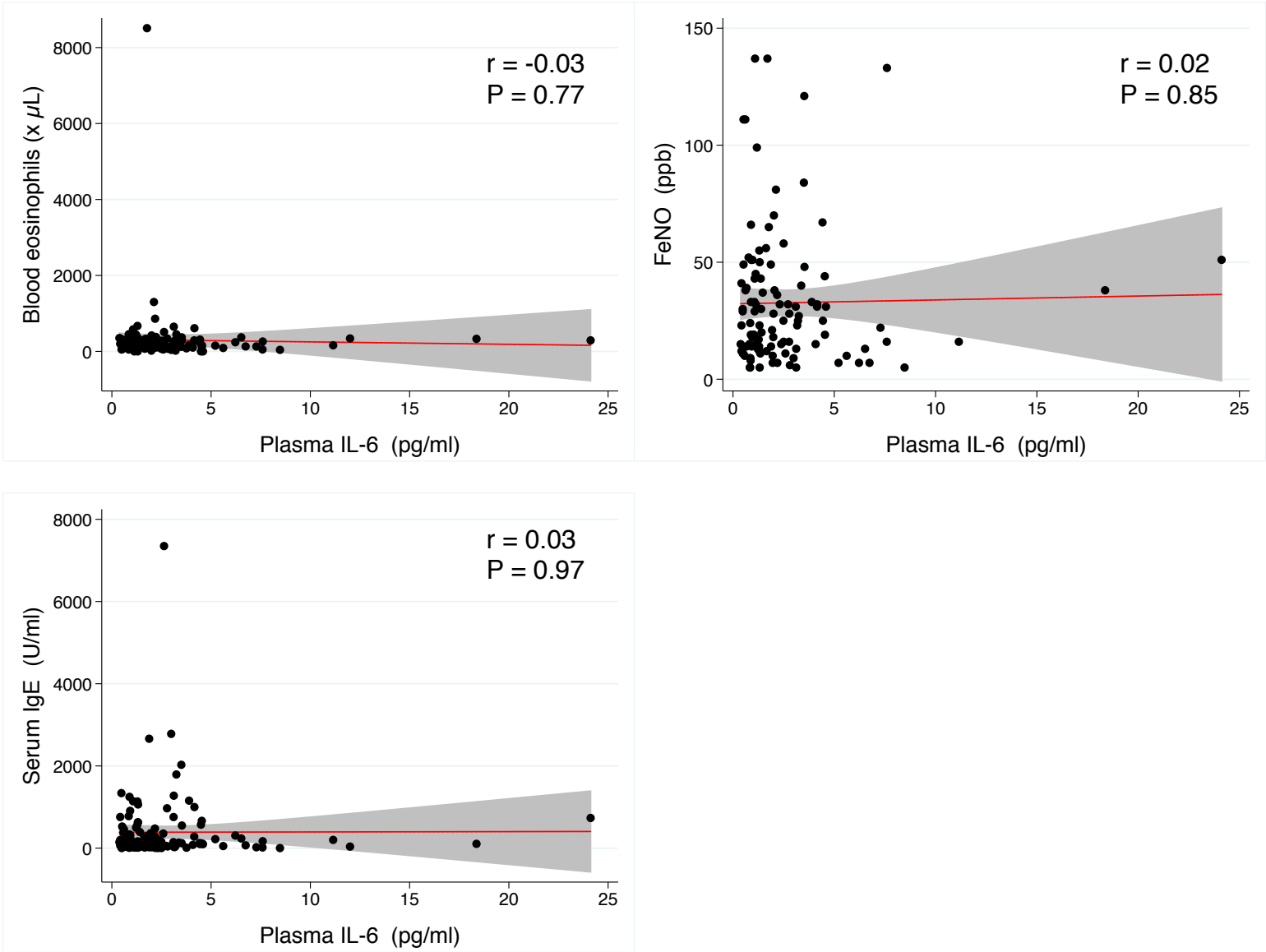


Figure S3



Online Repository

Evidence for an IL-6 high asthma phenotype in asthma patients of African ancestry

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Data analysis and interpretation: SW, BL, CO

Statistical analysis: SW, BL

Drafting and editing manuscript for important intellectual content: SW, BL

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Running title: IL-6 high phenotype in African-Americans with asthma

Supplemental Methods

Subjects. We conducted a prospective, observational clinical study that was approved by the Institutional Review Board at the University of Chicago. All subjects provided written, informed consent for participation. Subjects were recruited between July 1, 2011, and June 30, 2016. The subjects with asthma were recruited so as to provide a wide range of disease state from mild (no use of inhaled or oral corticosteroid), to a requirement for inhaled corticosteroid for disease control, to a requirement for oral corticosteroids for complete or incomplete disease control. Data from subsets of these subjects have previously been reported (1-7).

All clinical data and blood samples were collected at a single visit. Asthmatic subjects withheld the use of long-acting beta-adrenergic agonists for 24 hours prior to the first visit, at which time medical history, medication use and asthma questionnaires were administered; spirometry to measure FVC and FEV₁, methacholine challenge or reversibility studies as indicated, and measurement of exhaled nitric oxide (FeNO) were performed. Blood was collected for a complete blood count with cell differential and measurement of serum total IgE. Additional blood was collected into tubes containing ethylenediaminetetraacetic acid; plasma was separated by centrifugation at 4°C and then stored in aliquots at -80°C until analysis. Allergic sensitization was measured in all subjects either by skin prick testing for common aeroallergens, along with histamine and negative controls, or RAST testing for common aeroallergens. A positive wheal (> 3 mm) on skin-prick testing or specific IgE above the threshold for a grade 2 (low positive, out of grades 0 – 6) on RAST testing was scored as “positive” for that allergen. Positive results on skin prick or RAST testing were used interchangeably in data analysis.

Methacholine challenge testing was performed on all subjects when baseline predicted FEV₁ was ≥60%; reversibility after inhalation of albuterol was measured when baseline predicted FEV₁ was <60%, per guidelines of the American Thoracic Society (8). Subjects underwent all studies at a time of clinical stability and at least eight weeks after any known asthma exacerba-

tion or respiratory viral illness. Subjects with asthma met criteria defined by the *EPR 3 Guidelines on Asthma* (9). Control subjects had no lifetime history of pulmonary disease, were in good health, did not use respiratory-related medication, and had <16% reduction in FEV1 after inhalation of 25 mg/ml methacholine. Subjects with a smoking history of ≥ 10 pack/years or who were actively smoking within 6 months of potential recruitment, who had a history of chronic obstructive pulmonary disease, allergic bronchopulmonary aspergillosis, Churg-Strauss syndrome, or who had any medical contraindication to bronchoscopy were excluded.

Statistical analysis. Statistical analyses were conducted in STATA 14 (StataCorp, College Station, TX). Continuous variables are expressed as the mean $\pm$ SD or as median (IQR). Categorical values are presented as numeric data. Statistical analysis of clinical and demographic data was done using paired t-tests or by F-tests followed by t-tests with correction using the Bonferroni method as appropriate. Non-parametric tests were substituted (Kruskal-Wallis test followed by Mann-Whitney rank-sum test with correction for multiple comparisons) when a normal distribution could not be assumed. Linear regression was done using STATA; non-parametric correlation was assessed by Spearman's coefficient (ρ) and significance was determined using the Kendall rank correlation coefficient with continuity correction.

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Supplemental Figure Legends

Figure S1. A. Distribution of plasma IL-6 concentrations (pg/ml) in 68 control subjects and 124 subjects with mild to severe asthma. The dashed red line notes the threshold value for the entire control population. The gray box denotes the subjects with asthma with IL-6 concentrations above the threshold. $P < 0.05$ for asthma versus control subjects by Student t test. **B.** Distribution of plasma IL-6 concentrations (pg/ml) in 68 control subjects based on ancestry: 27 of European ancestry (EA), 32 of African ancestry (AA), and 9 of other ancestry. $P < 0.05$ for EA versus AA subjects by Student t test. Comparisons to the subjects in the other ancestry group were not made.

Figure S2. Body mass index in control subjects and subjects with mild to severe asthma. **A.** Number of control and asthmatic subjects with a BMI $> 30 \text{ kg/m}^2$. (left panel) **B.** Number of asthmatic subjects with plasma concentrations of IL-6 above and below the threshold, separated by body mass index above or below 30 kg/m^2 . **C.** Correlation between body mass index and plasma IL-6 concentrations in control subjects and subjects with mild to severe asthma. The gray area in each graph shows the 95% confidence interval of the correlation. Non-parametric correlation and significance were assessed by the Pearson coefficient (r).

Figure S3. Correlation between plasma IL-6 concentrations and number of blood eosinophils, fraction of exhaled nitric oxide (FeNO), and serum IgE in subjects with mild to severe asthma. In each graph, the gray area shows the 95% confidence interval of the correlation. Non-parametric correlation and significance were assessed by the Pearson coefficient (r). No significant correlations were noted.