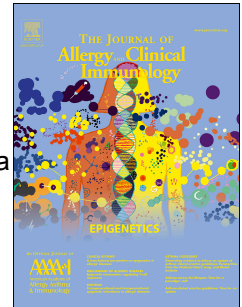


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Investigation of the relationship between IL-6 and type 2 biomarkers in severe asthma

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Key words: asthma severity; blood eosinophil count; FeNO; IL-6; corticosteroid responsiveness

Capsule Summary

Combination of IL-6 (non-Type 2 asthma) and FeNO or blood eosinophil count (Type 2 asthma) identified asthma endotypes related to asthma severity, exacerbations, and responsiveness to corticosteroids and potential for response to anti-Type 2 and anti-IL-6 treatment.

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Disclosure of potential conflict of interest:

A. T. Hastie reports grants from NIH and grant support from Genentech during the conduct of the study. W. W. Busse has received consulting fees from AstraZeneca, Genentech, Regeneron, Novartis, Sanofi, and GlaxoSmithKline. M. Castro receives University Grant Funding from NIH, American Lung Association, and PCORI, receives Pharmaceutical Grant Funding from AstraZeneca, Chiesi, Novartis, GSK, and Sanofi-Aventis, serves as a consultant for Genentech, Theravance, VIDA, Teva, Sanofi-Aventis and also a speaker for AstraZeneca, Genentech, GSK, Regeneron, Sanofi, Teva, and receives Royalties from Elsevier. S. E. Wenzel has consulted for AstraZeneca, Genentech, GSK, Sanofi-Aventis in the last 3 yrs, in matters unrelated to the content of this manuscript, participated in multicenter clinical trial for AstraZeneca, GSK, Novartis and Sanofi Aventis in the last 3 yrs, unrelated to this manuscript, and received financial support for the last 2 yrs of SARP from an unrestricted grant from Boehringer-Ingelheim. E. R. Bleecker has performed clinical trials through his employer, Wake Forest School of Medicine and University of Arizona, for AstraZeneca, MedImmune, Boehringer Ingelheim, Genentech, Novartis, Regeneron, and Sanofi Genzyme, and has also served as a paid consultant for ALK-Abello, AstraZeneca, MedImmune, Glaxo Smith Kline, Novartis, Regeneron, Sanofi Genzyme,

68 and TEVA, outside the submitted work. The rest of the authors declare that they have no relevant
69 conflict of interest.

To the Editor:

Asthma is a heterogeneous respiratory disease. Eosinophilic inflammation is important in Type 2 early onset more allergic asthma while neutrophilic inflammation or other unknown mechanisms are involved in non-Type 2 asthma. Identification of asthma endotypes linking biological pathways and molecular mechanisms for asthma phenotypes should lead to more precise approaches to characterize and treat asthma.

The NHLBI Severe Asthma Research Program (SARP) has an overall goal to identify clinical and pathophysiologic differences between mild-moderate and severe asthma. SARP1-2 were cross sectional studies and SARP3 was a longitudinal study with higher percentage of severe asthma compared to SARP1-2. In a previous genetic study in SARP1-2, the C allele of rs2228145 (Asp358Ala) in *IL6R* was associated with higher serum soluble IL-6R (sIL-6R) levels, asthma, and lower lung function.¹ Higher levels of plasma interleukin-6 (IL-6) have been correlated with higher BMI, lower lung function, and more frequent asthma exacerbations in the SARP3 and UCSF cohorts.² Higher fractional exhaled nitric oxide (FeNO) levels correlate with Type 2 asthma and better responses to corticosteroids.³ In this study, we investigated whether IL-6, sIL-6R, FeNO, blood eosinophil count and the combination of FeNO or blood eosinophil count (Type 2 asthma) and IL-6 (non-Type 2 asthma) levels are associated with clinical and cellular phenotypes in the SARP3 and SARP1-2 cohorts (See additional information on methods in the Online Repository). The large comprehensively phenotyped SARP database is ideal to examine relationship between Type 2 eosinophilic and non-Type 2 mechanisms in severe and non-severe asthma.

Adults (age $\geq$ 12 years) with asthma in SARP3 were older ($p<0.0001$) and with lower percentage of African Americans ($p<0.0001$) than SARP1-2 subjects (Table E1). With adjustment of age, sex, BMI, and race, SARP3 subjects had significantly higher percentage of severe asthma and systemic corticosteroid use in last year ($p<0.0001$), higher percentage of sputum neutrophils ($p=0.0002$), and lower baseline FEV₁/FVC ($p<0.0001$) than SARP1-2 subjects. SARP3 and SARP1-2 had similar levels of FeNO, blood eosinophil count, serum sIL-6R levels, and plasma or serum IL-6 levels ($p>0.05$), and thus a merged SARP1-3 dataset was used for analyses.

Serum sIL-6R levels were positively correlated with blood neutrophil counts ($p=0.0004$) but not significantly correlated with asthma severity or asthma exacerbations with adjustment for age, sex, BMI, and race (Table E2). We confirmed that the C allele of rs2228145 was associated with higher serum sIL-6R levels ($p<0.0001$). Interestingly, the C allele of rs2228145 was the risk allele for asthma, hay fever, and atopic dermatitis, but it has been reported to be the protective allele for rheumatoid arthritis.⁴ In summary, sIL-6R levels may not be a predictive biomarker for severe asthma and exacerbations, and thus were not used for further analyses in this study.

Higher plasma or serum IL-6 levels were significantly correlated with older age ($p=0.001$), female gender ($p=0.01$), and higher BMI ($p<0.0001$) in merged SARP1-3 dataset (Table E3) or with similar trends in SARP1-2 (Table E4) and SARP3 (Table E5). In a recent study, White et al. has reported that plasma IL-6 levels are higher in African Americans than Whites in both control and asthma groups.⁵ In this study, IL-6 levels were not significantly different in subjects with asthma between African Americans (2.7 ± 4.1 pg/ml; $n=230$) and Whites (2.6 ± 4.7 pg/ml; $n=476$) (Table E6). With adjustment for age, sex, BMI, and race, higher IL-6 levels were still

significantly correlated with lower baseline % predicted FEV₁ (p=0.01), higher blood neutrophil counts (p<0.0001), asthma hospitalizations in the last year (p=0.01), and systemic corticosteroid use in the last year (p<0.0001) in merged SARP1-3 dataset. IL-6 levels were not correlated with cellular characteristics of Type 2 asthma (higher blood eosinophil counts and % sputum eosinophils, total serum IgE levels, and FeNO levels) in merged SARP1-3 dataset (Table E3). Similar trends were observed in SARP1-2 (Table E4) and SARP3 (Table E5). In SARP1-3 subjects (n=695) with IL-6 levels, FeNO levels, and blood eosinophil counts, IL-6 levels and FeNO levels were independent (High IL-6 & High FeNO+Low IL-6 & Low FeNO:High IL-6 & Low FeNO+Low IL-6 & High FeNO=365:330) and IL-6 levels and blood eosinophil counts were also independent (HH+LL:HL+LH=390:305) (Figure 1). IL-6 levels were not significantly correlated with responses to corticosteroid evoked phenotypes⁶ (change of ACQ6 or % change of FEV₁) before and 3 weeks after an intramuscular triamcinolone acetonide injection in SARP3. In summary, IL-6 levels may be a useful biomarker associated with severe asthma and exacerbations. Thus, IL-6 levels are correlated with asthma phenotypes but not Type 2 asthma biomarkers.

Higher FeNO levels were significantly correlated with younger age (p=0.02) and lower BMI (p=0.0008) (Table E7). With adjustment for age, sex, BMI, and race, higher FeNO levels were still significantly correlated with lower baseline % predicted FEV₁ (p=0.002) and FEV₁/FVC (p<0.0001), greater bronchodilator reversibility (p<0.0001), asthma hospitalizations in the last year (p=0.05), ICU admissions due to asthma in the last year (p=0.02), and systemic corticosteroid use in the last year (p=0.03). FeNO levels were positively correlated with cellular characteristics of Type 2 asthma (higher blood eosinophil counts, % sputum eosinophils, and

total serum IgE levels) ($p < 0.0001$). In SARP1-3 subjects ($n = 695$), FeNO levels were positively correlated with blood eosinophil counts (HH+LL:HL+LH=448:247) (Figure 1). Higher FeNO levels were significantly correlated with greater % change of FEV₁ ($p = 0.0004$) and greater decrease of ACQ6 ($p = 0.01$) before and 3 weeks after an intramuscular triamcinolone acetonide injection. With adjustment for age, sex, BMI, and race, higher blood eosinophil count was significantly correlated with lower baseline % predicted FEV₁ ($p < 0.0001$) and FEV₁/FVC ($p < 0.0001$), greater bronchodilator reversibility ($p < 0.0001$), and higher systemic corticosteroid use in last year ($p = 0.03$) (Table E8). Blood eosinophil count was positively correlated with cellular characteristics of Type 2 asthma (higher % sputum eosinophils, FeNO, and total serum IgE levels) ($p < 0.0001$). Higher blood eosinophil count was significantly correlated with greater % change of FEV₁ ($p = 0.0004$) before and 3 weeks after an intramuscular triamcinolone acetonide injection. In summary, FeNO levels and blood eosinophil count may be useful biomarkers for severe asthma and exacerbations. FeNO levels and blood eosinophil count are correlated with asthma phenotypes and corticosteroid evoked phenotypes representing Type 2 asthma biomarkers.

Phenotypes were compared among four groups categorized by the combinations of High-Low IL-6 levels (≥ 3.1 pg/ml or < 3.1 pg/ml) and High-Low FeNO levels (≥ 25 ppb or < 25 ppb) (Table I and Table E9-E11) or High-Low blood eosinophil count (≥ 300 cells/ μ l or < 300 cells/ μ l) (Table I and Table E12). Two high IL-6 groups (HL and HH) had higher blood neutrophil counts ($p < 0.0001$), higher BMI ($p < 0.0001$), lower baseline % predicted FEV₁ ($p = 0.01$), higher percentage of asthma hospitalizations ($p = 0.02$) and systemic corticosteroid use in last year ($p = 0.005$) than two low IL-6 groups (LL and LH) in merged SARP1-3 dataset (Table E9), and

showed similar trend in SARP1-2 (Table E10) and SARP3 (Table E11). Two high FeNO groups (LH and HH) had higher sputum and blood eosinophils and total serum IgE levels ($p<0.0001$), lower baseline FEV₁/FVC ($p=0.02$), greater bronchodilator reversibility ($p=0.004$), and greater % changes of FEV₁ before and after corticosteroid administration⁶ ($p=0.04$) than two low FeNO groups (LL and HL) in merged SARP1-3 dataset (Table E9). Two high blood eosinophil count groups (LH and HH) had higher sputum eosinophils, FeNO, and total serum IgE levels ($p<0.0001$), lower FEV₁/FVC ($p=0.01$), greater bronchodilator reversibility ($p=0.004$), and greater % changes of FEV₁ before and after corticosteroid administration ($p=0.004$) than two low blood eosinophil count groups (HL and LL) (Table E12). High FeNO and high blood eosinophil count group (HH) or low FeNO and low blood eosinophil count group (LL) had greater or lower % changes of FEV₁ before and after corticosteroid administration or % sputum eosinophils than high FeNO and low blood eosinophil count group (HL) or low FeNO and high blood eosinophil count group (LH), respectively ($p<0.0001$) (Table E13). Combinations of FeNO and blood eosinophil count appear to be a better predictive biomarker for eosinophilic inflammation than FeNO or blood eosinophil count. In summary, subjects with higher IL-6 levels tend to be more obese with higher blood neutrophil counts, more severe asthma and asthma exacerbations; subjects with higher FeNO levels or blood eosinophil count tend to have Type 2 asthma with eosinophilic inflammation, more severe asthma and asthma exacerbations, and larger improvement after systemic corticosteroid administration. These four groups may reflect four different asthma endotypes.

Previous studies have indicated that FeNO is a reliable non-invasive biomarker of eosinophilic inflammation and corticosteroid responses.³ IL-6 binding to membrane IL-6R (classic IL-6

signaling pathway) or sIL-6R (trans-signaling pathway) is involved in host defence against infections, asthma, autoimmune diseases, and cardiovascular diseases.^{7,8} Combination of sputum eosinophils and neutrophils may identify subjects with more severe asthma symptoms.⁹ The distribution of IL-6 levels, FeNO levels, and blood eosinophil counts were continuous (Figure E1-E2), indicating that the choice of cutoff values was relatively subjective. Identification of the best cutoff values for these biomarkers is warranted for future categorization of asthma endotypes. Correlation of biomarkers (as quantitative variables) was performed using Spearman rank correlation (Table E14). IL-6 levels were not significantly correlated with Type 2 asthma biomarkers, and thus should reflect a non-Type 2 asthma. IL-6 levels were positively correlated with blood neutrophil count ($p < 0.0001$) but not significantly correlated with % sputum neutrophils, which indicate IL-6 levels may not be a good surrogate for airway neutrophilic inflammation. Type 2 asthma biomarkers (% sputum eosinophils, blood eosinophil count, FeNO, and total serum IgE levels) were positively correlated with each other ($p < 0.0001$), but the correlation was not strong ($0.2 < \text{correlation coefficient } \rho < 0.5$), which may indicate there are more than one Type 2 asthma endotype.

In this study, we showed that combination of IL-6 (representing non-Type 2 asthma) and FeNO or blood eosinophil count (representing Type 2 asthma) may identify different asthma endotypes (Figure E3). In the future, it is important to test measurement consistency, longitudinal changes, and drug effects on these biomarkers. It will be also important to evaluate treatment with IL-6 antibodies to determine their effects on asthma severity phenotypes and control. For example, we can postulate that a combination of anti-Type 2 and anti-IL-6 drugs may be needed for treating severe asthmatics with the HH endotype, while anti-Type 2 or anti-IL-6 therapies may be

effective in severe asthmatics with the LH or HL endotypes, respectively. Additional forms of therapies may be more effective for the LL endotype. It will be critical to develop and test potential biomarkers that will predict responsiveness or lack of responsiveness to the biologic therapies, in order to advance stratified therapy in asthma.

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Table I. Correlation of IL-6 and FeNO levels or blood eosinophil count with asthma phenotypes in SARP1-3 (partial table).

IL-6 & FeNO	LL (278)*		LH (249)*		HL (88)*		HH (90)*		Four Groups	
	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Body mass index (BMI)	278	31 $\pm$ 6.9	249	29 $\pm$ 6.9	88	39 $\pm$ 12	90	35 $\pm$ 9.0	<0.0001	NA
Baseline FEV ₁ % predicted	278	77 $\pm$ 21	249	76 $\pm$ 19	88	70 $\pm$ 21	90	70 $\pm$ 19	0.001	0.014
Blood neutrophil counts (cells/ μ l)	273	3974 $\pm$ 1625	247	3822 $\pm$ 1562	86	5576 $\pm$ 2407	87	5379 $\pm$ 3133	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	273	201 $\pm$ 158	245	339 $\pm$ 286	85	223 $\pm$ 262	86	345 $\pm$ 270	<0.0001	<0.0001
FeNO (ppb)	278	14 $\pm$ 5.4	249	58 $\pm$ 41	88	15 $\pm$ 5.7	90	49 $\pm$ 35	<0.0001	<0.0001
Systemic corticosteroid use in last year (% yes)	278	28	249	27	88	45	90	50	<0.0001	0.0048
% Δ FEV ₁ (corticosteroid evoked) in SARP3	145	2.6 $\pm$ 13	107	6.8 $\pm$ 16	48	1.4 $\pm$ 15	40	8.8 $\pm$ 18	0.0007	0.039
IL-6 & Blood eosinophil count	LL (344)**		LH (216)**		HL (113)**		HH (65)**		Four Groups	
	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Body mass index (BMI)	344	30 $\pm$ 7.0	215	30 $\pm$ 7.1	113	38 $\pm$ 11	65	35 $\pm$ 9.3	<0.0001	NA
Baseline FEV ₁ % predicted	344	78 $\pm$ 20	215	73 $\pm$ 19	113	71 $\pm$ 19	65	68 $\pm$ 20	<0.0001	0.0033
Blood neutrophil counts (cells/ μ l)	344	3818 $\pm$ 1694	216	4003 $\pm$ 1459	113	5320 $\pm$ 2800	65	5578 $\pm$ 2631	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	344	136 $\pm$ 70	216	480 $\pm$ 262	113	141 $\pm$ 80	65	524 $\pm$ 305	<0.0001	<0.0001
FeNO (ppb)	327	28 $\pm$ 29	197	47 $\pm$ 44	108	27 $\pm$ 23	63	41 $\pm$ 38	<0.0001	<0.0001
Systemic corticosteroid use in last year (% yes)	344	25	215	29	113	44	65	54	<0.0001	0.002
% Δ FEV ₁ (corticosteroid evoked) in SARP3	164	3.3 $\pm$ 13	92	6.2 $\pm$ 17	55	0.4 $\pm$ 11	33	12 $\pm$ 21	0.008	0.004

*LL: low IL-6 and low FeNO, LH: low IL-6 and high FeNO, HL: high IL-6 and low FeNO, HH: high IL-6 and high FeNO; †Kruskal-

Wallis test; ‡Generalized linear model adjusted for age, sex, BMI, and race; **LL: low IL-6 and low blood eosinophil count, LH: low

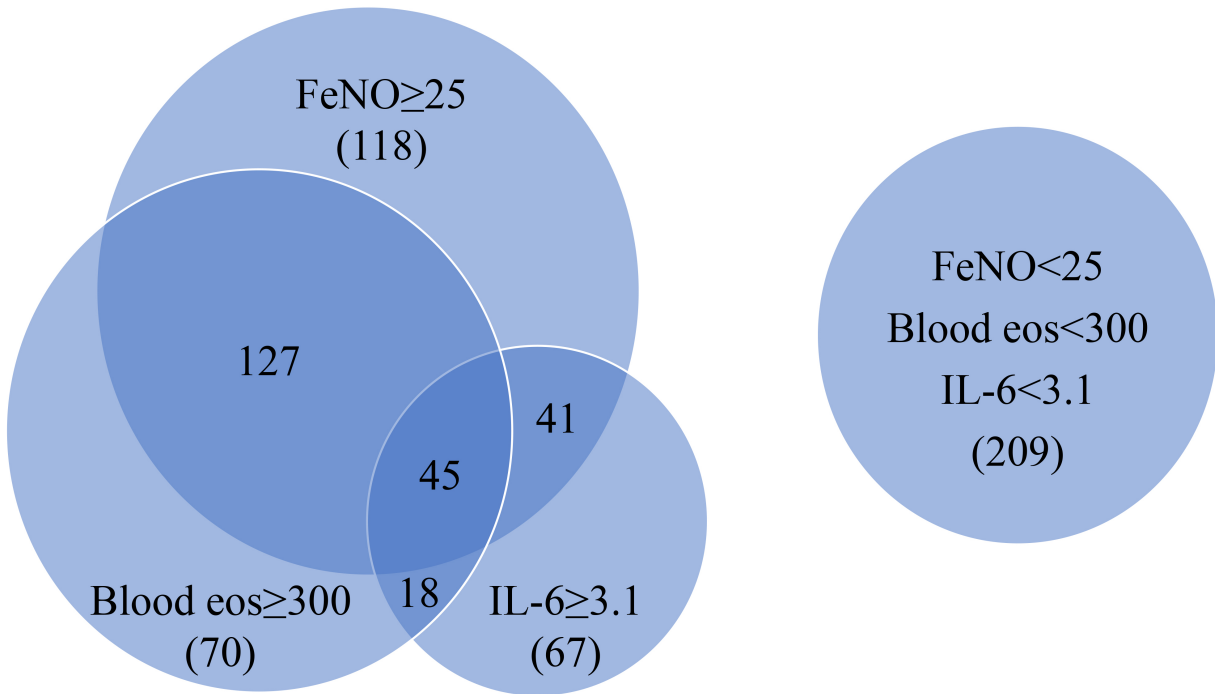
IL-6 and high blood eosinophil count, HL: high IL-6 and low blood eosinophil count, HH: high IL-6 and high blood eosinophil count.

This is a partial table for combination of IL-6 and FeNO levels or IL-6 and blood eosinophil count. Please refer to Table E9 and Table

E12 for complete phenotypes tested.

281 **Figure Legends**

282 **Figure 1.** Venn diagram of adults with asthma and IL-6 levels, FeNO levels, and blood
283 eosinophil counts in SARP1-3 (n=695). Subjects were categorized into high IL-6 (≥ 3.1 pg/ml,
284 or low IL-6 (< 3.1 pg/ml), high FeNO (≥ 25 ppb) or low FeNO (< 25 ppb), and high blood
285 eosinophils (≥ 300 cells/ μ l) or low blood eosinophils (< 300 cells/ μ l).



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Letters To The Editors

Investigation of the relationship between IL-6 and type 2 biomarkers in severe asthma

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Study Subjects

The Severe Asthma Research Program (SARP) is a National Heart, Lung, and Blood Institute (NHLBI)-sponsored project with an overall goal of identifying clinical and pathophysiologic differences between mild-moderate and severe asthma. SARP1 and 2 cohorts were cross-sectional studies from 2001 to 2012 (n~1,600) with the overall aim to study mechanisms differentiating severe from non-severe asthma.^{E1-E3} SARP3 cohort is an ongoing longitudinal study (n=714) with the main objective to advance understanding of severe asthma through the integration of mechanistic studies with detailed phenotypic characterization and molecular and genomic studies.^{E4-E7} To accomplish this goal, cohorts of non-smokers (<5 pack-years of tobacco use) with asthma (enriched for severe asthma) was recruited for detailed characterization and enrollment into an observational cross sectional (SARP1-2) or longitudinal (SARP3) study. SARP3 subjects were carefully and extensively phenotyped using procedures, adapted from SARP1-2 which included history and physical examination, questionnaires, asthma-related quality of life, spirometry, sputum induction, total serum IgE levels, fractional exhaled nitric oxide (FeNO), and corticosteroid evoked phenotypes before and 3 weeks after an intramuscular triamcinolone acetonide injection.^{E7} SARP3 was designed as a three-year longitudinal study and the cohort is still being followed. SARP1-3 studies were approved by the appropriate institutional review board at the participating sites including informed consent.

Genotyping

DNA was isolated using standard protocols, and SNP genotyping was performed using the Illumina HumanHap1M BeadChip. Genotyping call was performed using BeadStudio (Illumina,

Inc., San Diego, California). rs2228145, a missense variant in *IL6R* was extracted and the genotype was recoded (AA=0, CA=1, and CC=2) and analyzed using PLINK.^{E8}

IL-6 and sIL-6R measurement

In SARP3, plasma IL-6 protein levels of 387 adults (age $\geq$ 12 years) with asthma were measured as described.^{E9} In brief, plasma was treated with ethylenediaminetetraacetic acid and IL-6 levels were measured using high sensitivity human IL-6 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA). In SARP1-2, serum IL-6 protein levels of 376 adults with asthma were measured using high sensitivity human IL-6 kit similar to SARP3. In SARP1-3, serum soluble IL-6R (sIL-6R) levels were measured as described.^{E10} In brief, serum sIL-6R levels of 803 adults with asthma were measured with sIL-6R DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA).

Statistical Analysis

Unadjusted p-values were calculated using Kruskal-Wallis test for binary variables or Spearman Rank test for quantitative variables. Adjusted p-values (adjusted for age, sex, BMI, and race) were calculated using generalized linear models. Log-transformation was applied to IL-6 levels, sIL-6R levels, % sputum eosinophils and neutrophils, blood eosinophil and neutrophil counts, total serum IgE levels, and FeNO. Analyses of serum sIL-6R (n=803), FeNO (n=851), or blood eosinophil count (n=877) were performed in SARP1-3 merged dataset. Analyses of serum or plasma IL-6 (n=763) were performed in SARP1-3 merged dataset or SARP1-2 and SARP3 separate datasets. Analyses of two groups categorized by high IL-6 ($\geq$ 3.1 pg/ml, n=186) and low IL-6 ($<$ 3.1 pg/ml, n=577), by high FeNO ($\geq$ 25 ppb, n=409) and low FeNO ($<$ 25 ppb, n=442),

and by high blood eosinophil count (≥ 300 cells/ μ l, n=330) and low blood eosinophil count (< 300 cells/ μ l, n=547) or four groups categorized by the combinations of High-Low IL-6 levels (≥ 3.1 pg/ml or < 3.1 pg/ml) and High-Low FeNO levels (≥ 25 ppb or < 25 ppb), i.e., HH (High IL-6 & High FeNO; n=90), HL (High IL-6 & Low FeNO; n=88), LH (Low IL-6 & High FeNO; n=249), and LL (Low IL-6 & Low FeNO; n=278), by the combinations of High-Low IL-6 levels (≥ 3.1 pg/ml or < 3.1 pg/ml) and High-Low blood eosinophil count (≥ 300 cells/ μ l or < 300 cells/ μ l), i.e., HH (High IL-6 & High blood eosinophil; n=65), HL (High IL-6 & Low blood eosinophil; n=113), LH (Low IL-6 & High blood eosinophil; n=216), and LL (Low IL-6 & Low blood eosinophil; n=344), or by the combinations of High-Low FeNO levels (≥ 25 ppb or < 25 ppb) and High-Low blood eosinophil count (≥ 300 cells/ μ l or < 300 cells/ μ l), i.e., HH (High FeNO & High blood eosinophil; n=203), HL (High FeNO & Low blood eosinophil; n=195), LH (Low FeNO & High blood eosinophil; n=106), and LL (Low FeNO & Low blood eosinophil; n=327) were performed in SARP1-3. Correlation of biomarkers (% sputum eosinophils and neutrophils, blood eosinophil and neutrophil counts, FeNO, total serum IgE levels, and IL-6 levels) was tested using Spearman rank correlation. SAS 9.4 software (www.sas.com) was used for all statistical analyses. Nominal p-value of 0.05 was considered as significant.

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136 Allergy Clin Immunol. 2012;130(2):510-5 e1.

137 Table E1. Demographics (Mean $\pm$ SD) of adults with asthma and IL-6 levels in SARP1-2 and SARP3.

	SARP1-2 (n=376)		SARP3 (n=387)		P value†	P value‡ (Adjusted)
	n	Mean $\pm$ SD	n	Mean $\pm$ SD		
Age at enrollment, years	376	38 $\pm$ 14	387	47 $\pm$ 14	<0.0001	NA
Gender (% female)	376	70	387	66	0.28	NA
Race (% White/African American/others)	376	58/37/4	387	66/23/11	<0.0001	NA
Body mass index (BMI)	375	31 $\pm$ 8.7	387	32 $\pm$ 8.4	0.017	NA
Baseline FEV ₁ % predicted	375	77 $\pm$ 20	387	72 $\pm$ 20	0.0008	0.83
Baseline FEV ₁ /FVC	375	0.73 $\pm$ 0.11	387	0.68 $\pm$ 0.11	<0.0001	<0.0001
Maximal FEV ₁ % predicted	375	89 $\pm$ 17	386	84 $\pm$ 20	0.0002	0.80
Change in % predicted FEV ₁ to albuterol	375	17 $\pm$ 17	386	19 $\pm$ 18	0.016	0.17
% Sputum neutrophils	242	41 $\pm$ 26	293	55 $\pm$ 25	<0.0001	0.0002
% Sputum eosinophils	240	4.2 $\pm$ 8.5	293	4.2 $\pm$ 9.3	0.049	0.013
Blood neutrophil counts (cells/ μ l)	355	4106 $\pm$ 1987	387	4434 $\pm$ 2132	0.042	0.15
Blood eosinophil counts (cells/ μ l)	351	250 $\pm$ 210	387	292 $\pm$ 275	0.15	0.54
FeNO (ppb)	327	39 $\pm$ 39	384	30 $\pm$ 30	0.013	0.10
Total serum IgE (IU/ml)	344	348 $\pm$ 908	382	344 $\pm$ 582	0.13	0.0016
ATS classification (% severe asthma)	376	24	387	58	<0.0001	<0.0001
ER or urgent care visit for asthma in last year (% yes)	375	37	387	42	0.14	0.31
Systemic corticosteroid use in last year (% yes)	375	6	387	56	<0.0001	<0.0001
Hospitalization for breathing problem in last year (% yes)	374	7	387	12	0.017	0.05
ICU admission for asthma in last year (% yes)	375	3	387	3	1.0	0.75
Serum or Plasma IL-6 levels (pg/ml)*	376	2.7 $\pm$ 5.8	387	2.5 $\pm$ 2.2	0.0017	0.22
Serum sIL-6R levels (ng/ml)	374	39 $\pm$ 13	287	39 $\pm$ 9.4	0.50	0.38

138 *SARP1-2 serum IL-6 or SARP3 plasma IL-6; †Kruskal-Wallis test; ‡Generalized linear model adjusted for age, sex, BMI, and race.

139 Table E2. Correlation of serum sIL-6R levels with asthma phenotypes in SARP1-3.

	n	rho*	P value*	Adj. P value†
Age at enrollment, years	803	0.17	<0.0001	NA
Gender (% female)	803	0.009	0.37	NA
Race (% White/African American/others)	803	-0.04	<0.0001	NA
Body mass index (BMI)	802	0.07	0.043	NA
Baseline FEV ₁ % predicted	803	-0.07	0.049	0.81
Baseline FEV ₁ /FVC	803	0.017	0.63	0.095
Maximal FEV ₁ % predicted	800	-0.09	0.0124	0.8
Change in % predicted FEV ₁ to albuterol	800	-0.03	0.35	0.19
% Sputum neutrophils	566	-0.004	0.93	0.75
% Sputum eosinophils	564	0.04	0.34	0.053
Blood neutrophil counts (cells/ μ l)	780	0.19	<0.0001	0.0004
Blood eosinophil counts (cells/ μ l)	776	0.05	0.16	0.64
FeNO (ppb)	748	0.014	0.7	0.13
Total serum IgE (IU/ml)	762	-0.008	0.82	0.09
ATS classification (% severe asthma)	803	0.012	0.21	0.44
ER or urgent care visit for asthma in last year (% yes)	803	0.012	0.2	0.75
Systemic corticosteroid use in last year (% yes)	803	0.02	0.033	0.59
Hospitalization for breathing problem in last year(% yes)	801	0.004	0.81	0.94
ICU admission for asthma in last year (% yes)	802	-0.03	0.24	0.48
Serum IL-6 levels (pg/ml) in SARP1-2	374	0.16	0.0017	0.08
Plasma IL-6 levels (pg/ml) in SARP3	287	0.22	0.0002	0.04
<i>IL6R</i> rs2228145 (% AA/CA/CC)	762	0.11	<0.0001	<0.0001

140 *Spearman rank test correlation coefficient rho and p-value; †Generalized linear model adjusted for age, sex, BMI, race.

141 Table E3. Correlation of IL-6 levels with asthma phenotypes in SARP1-3.

	IL-6 Low (< 3.1 pg/ml)		IL-6 High (≥ 3.1 pg/ml)		P value†	P value‡ (Adjusted)
	n	Mean $\pm$ SD	n	Mean $\pm$ SD		
Age at enrollment, years	577	42 $\pm$ 15	186	46 $\pm$ 14	0.0014	NA
Gender (% female)	577	65	186	75	0.012	NA
Race (% White/African American/others)	577	63/30/7	186	61/31/8	0.91	NA
Body mass index (BMI)	576	30 $\pm$ 7.1	186	37 $\pm$ 10	<0.0001	NA
Baseline FEV ₁ % predicted	576	76 $\pm$ 20	186	69 $\pm$ 20	<0.0001	0.01
Baseline FEV ₁ /FVC	576	0.70 $\pm$ 0.11	186	0.70 $\pm$ 0.11	0.7	0.9
Maximal FEV ₁ % predicted	576	88 $\pm$ 18	185	81 $\pm$ 18	<0.0001	0.02
Change in % predicted FEV ₁ to albuterol	576	17 $\pm$ 17	185	20 $\pm$ 19	0.1	0.2
% Sputum neutrophils	401	48 $\pm$ 27	134	51 $\pm$ 27	0.14	0.35
% Sputum eosinophils	400	4.4 $\pm$ 9.5	133	3.8 $\pm$ 6.8	0.58	0.15
Blood neutrophil counts (cells/ μ l)	562	3898 $\pm$ 1615	180	5462 $\pm$ 2772	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	560	269 $\pm$ 240	178	281 $\pm$ 268	0.85	0.59
FeNO (ppb)	533	35 $\pm$ 36	178	32 $\pm$ 30	0.85	0.11
Total serum IgE (IU/ml)	548	342 $\pm$ 809	178	357 $\pm$ 551	0.65	0.15
ATS classification (% severe asthma)	577	38	186	52	0.0006	0.1
ER or urgent care visit for asthma in last year (% yes)	576	36	186	50	0.0019	0.1
Systemic corticosteroid use in last year (% yes)	576	26	186	48	<0.0001	<0.0001
Hospitalization for breathing problem in last year (% yes)	575	7	186	16	0.0013	0.014
ICU admission for asthma in last year (% yes)	576	3	186	4	0.33	0.4
Serum or Plasma IL-6 levels (pg/ml)*	577	1.4 $\pm$ 0.72	186	6.5 $\pm$ 7.5	<0.0001	<0.0001
sIL-6R levels (ng/ml)	505	38 $\pm$ 12	156	42 $\pm$ 12	0.001	0.03
IL6R rs2228145 (% AA/CA/CC)	528	49/37/14	174	45/39/16	0.71	0.12
% Δ FEV ₁ (corticosteroid evoked) in SARP3	256	4.3 $\pm$ 14	88	4.8 $\pm$ 17	0.66	0.2
Δ ACQ6 (corticosteroid evoked) in SARP3	264	-0.17 $\pm$ 0.74	93	-0.31 $\pm$ 0.77	0.2	0.15

142 *SARP1-2 serum IL-6 or SARP3 plasma IL-6; †Kruskal-Wallis test; ‡Generalized linear model adjusted for age, sex, BMI, and race.

143 Table E4. Correlation of IL-6 levels with asthma phenotypes in SARP1-2.

	IL-6 Low (<3.1 pg/ml)		IL-6 High (≥ 3.1 pg/ml)		P value*	P value† (Adjusted)
	n	Mean $\pm$ SD	n	Mean $\pm$ SD		
Age at enrollment, years	293	37 $\pm$ 14	83	41 $\pm$ 14	0.015	NA
Gender (% female)	293	69	83	72	0.59	NA
Race (% Caucasian/African American/others)	293	57/39/4	83	65/33/2	0.38	NA
Body mass index (BMI)	292	30 $\pm$ 7.4	83	36 $\pm$ 11	<0.0001	NA
Baseline FEV ₁ % predicted	292	78 $\pm$ 19	83	73 $\pm$ 20	0.059	0.28
Baseline FEV ₁ /FVC	292	0.73 $\pm$ 0.1	83	0.73 $\pm$ 0.11	0.59	0.79
Maximal FEV ₁ % predicted	292	90 $\pm$ 17	83	84 $\pm$ 18	0.008	0.18
Change in % predicted FEV ₁	292	17 $\pm$ 18	83	16 $\pm$ 16	0.84	0.73
% Sputum neutrophils	184	40 $\pm$ 26	58	43 $\pm$ 25	0.26	0.36
% Sputum eosinophils	183	4.1 $\pm$ 8.7	57	4.8 $\pm$ 7.9	0.19	0.15
Blood neutrophil counts (cells/ μ l)	278	3803 $\pm$ 1469	77	5200 $\pm$ 2996	<0.0001	0.0004
Blood eosinophil counts (cells/ μ l)	276	250 $\pm$ 187	75	247 $\pm$ 280	0.28	0.018
FeNO (ppb)	252	39 $\pm$ 41	75	36 $\pm$ 34	0.72	0.62
Total serum IgE (IU/ml)	268	379 $\pm$ 1000	76	238 $\pm$ 441	0.015	0.56
ATS classification (% severe asthma)	293	22	83	30	0.14	0.72
ER or urgent care visit for asthma in last year (% yes)	292	34	83	47	0.039	0.09
Systemic corticosteroid use (% yes)	292	4	83	13	0.0086	0.035
ER visit for breathing problem (% yes)	290	22	83	24	0.77	0.93
Hospitalization for breathing problem (% yes)	291	6	83	10	0.22	0.38
ICU admission for asthma in last year (% yes)	292	3	83	4	0.73	0.73
Serum IL-6 levels (pg/ml)	293	1.3 $\pm$ 0.75	83	7.8 $\pm$ 10.9	<0.0001	<0.0001
Serum sIL-6R levels (ng/ml)	291	38 $\pm$ 13	83	42 $\pm$ 14	0.03	0.18
<i>IL6R</i> rs2228145 (% AA/CA/CC)	278	53/34/13	81	42/40/18	0.17	0.09

144 *Kruskal-Wallis test; †Generalized linear model adjusted for age, sex, BMI, and race.

145 Table E5. Correlation of IL-6 levels with asthma phenotypes in SARP3.

	IL-6 Low (< 3.1 pg/ml)		IL-6 High (≥ 3.1 pg/ml)		P value*	P value† (Adjusted)
	n	Mean $\pm$ SD	n	Mean $\pm$ SD		
Age at enrollment, years	284	46 $\pm$ 14	103	49 $\pm$ 13	0.095	NA
Gender (% female)	284	62	103	78	0.0035	NA
Race (% Caucasian/African American/others)	284	69/21/10	103	58/29/13	0.15	NA
Body mass index (BMI)	284	30 $\pm$ 6.8	103	38 $\pm$ 9.8	<0.0001	NA
Baseline FEV ₁ % predicted	284	74 $\pm$ 20	103	66 $\pm$ 19	0.0006	0.0092
Baseline FEV ₁ /FVC	284	0.68 $\pm$ 0.11	103	0.68 $\pm$ 0.11	0.5	0.53
Maximal FEV ₁ % predicted	284	86 $\pm$ 20	102	79 $\pm$ 19	0.0022	0.053
Change in % predicted FEV ₁	284	18 $\pm$ 17	102	22 $\pm$ 20	0.03	0.025
% Sputum neutrophils	217	54 $\pm$ 25	76	58 $\pm$ 26	0.32	0.43
% Sputum eosinophils	217	4.6 $\pm$ 10.2	76	3.0 $\pm$ 5.9	0.8	0.39
Blood neutrophil counts (cells/ μ l)	284	3991 $\pm$ 1743	103	5658 $\pm$ 2590	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	284	287 $\pm$ 281	103	306 $\pm$ 257	0.28	0.15
FeNO (ppb)	281	31 $\pm$ 31	103	29 $\pm$ 28	0.41	0.09
Total serum IgE (IU/ml)	280	307 $\pm$ 569	102	446 $\pm$ 608	0.0084	0.009
ATS classification (% severe asthma)	284	54	103	70	0.0051	0.09
ER or urgent care visit for asthma in last year (% yes)	284	39	103	51	0.027	0.37
Systemic corticosteroid use (% yes)	284	48	103	76	<0.0001	0.0002
Hospitalization for breathing problem (% yes)	284	9	103	20	0.0039	0.012
ICU admission for asthma in last year (% yes)	284	2	103	5	0.32	0.29
Plasma IL-6 levels (pg/ml)	284	1.4 $\pm$ 0.69	103	5.5 $\pm$ 2.3	<0.0001	<0.0001
Serum sIL-6R levels (ng/ml)	214	38 $\pm$ 9.3	73	41 $\pm$ 9.3	0.0085	0.19
IL6R rs2228145 (% AA/CA/CC)	250	44/40/16	93	48/38/14	0.75	0.67
% Δ FEV ₁ (corticosteroid evoked) in SARP3	256	4.3 $\pm$ 14	88	4.8 $\pm$ 17	0.66	0.2
Δ ACQ6 (corticosteroid evoked) in SARP3	264	-0.17 $\pm$ 0.74	93	-0.31 $\pm$ 0.77	0.2	0.15

146 *Kruskal-Wallis test; †Generalized linear model adjusted for age, sex, BMI, and race.

147 Table E6. Demographics (Mean $\pm$ SD) of adults with asthma and IL-6 levels in Whites and African Americans.

	White		African American		P value†	P value‡ (Adjusted)
	n	Mean $\pm$ SD	n	Mean $\pm$ SD		
Age at enrollment, years	476	45 $\pm$ 14	230	38 $\pm$ 14	<0.0001	NA
Gender (% female)	476	67	230	70	0.61	NA
Body mass index (BMI)	475	31 $\pm$ 7.9	230	34 $\pm$ 9.7	0.0001	NA
Baseline FEV ₁ % predicted	475	75 $\pm$ 20	230	74 $\pm$ 20	0.54	0.022
Baseline FEV ₁ /FVC	475	0.70 $\pm$ 0.11	230	0.70 $\pm$ 0.11	0.78	0.02
Maximal FEV ₁ % predicted	474	86 $\pm$ 19	230	87 $\pm$ 18	0.45	0.40
Change in % predicted FEV ₁ to albuterol	474	16 $\pm$ 16	230	21 $\pm$ 20	0.003	0.007
% Sputum neutrophils	344	49 $\pm$ 27	143	47 $\pm$ 27	0.32	0.35
% Sputum eosinophils	342	3.8 $\pm$ 8.4	143	4.6 $\pm$ 9.1	0.06	0.027
Blood neutrophil counts (cells/ μ l)	463	4462 $\pm$ 1917	222	3954 $\pm$ 2455	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	461	282 $\pm$ 262	220	249 $\pm$ 209	0.27	0.52
FeNO (ppb)	451	32 $\pm$ 33	206	39 $\pm$ 40	0.11	0.05
Total serum IgE (IU/ml)	450	239 $\pm$ 474	220	536 $\pm$ 1138	<0.0001	<0.0001
ATS classification (% severe asthma)	476	42	230	38	0.33	0.93
ER or urgent care visit for asthma in last year (% yes)	475	40	230	42	0.57	0.55
Systemic corticosteroid use in last year (% yes)	475	34	230	25	0.012	0.07
Hospitalization for breathing problem in last year (% yes)	475	9	230	10	0.49	0.47
ICU admission for asthma in last year (% yes)	475	2	230	6	0.006	0.015
Serum or Plasma IL-6 levels (pg/ml) in SARP1-3*	476	2.6 $\pm$ 4.7	230	2.7 $\pm$ 4.1	0.93	0.54
Serum IL-6 levels (pg/ml) in SARP1-2	221	2.8 $\pm$ 6.5	141	2.6 $\pm$ 4.8	0.69	0.62
Plasma IL-6 levels (pg/ml) in SARP3	255	2.4 $\pm$ 2.2	89	2.8 $\pm$ 2.4	0.20	0.92
sIL-6R levels (ng/ml)	414	42 $\pm$ 12	197	33 $\pm$ 9.7	<0.0001	<0.0001
IL6R rs2228145 (% AA/CA/CC)	451	37/43/20	200	74/24/2	<0.0001	<0.0001
% Δ FEV ₁ (corticosteroid evoked) in SARP3	233	3.7 $\pm$ 15	74	5.7 $\pm$ 16	0.30	0.12
Δ ACQ6 (corticosteroid evoked) in SARP3	233	-0.19 $\pm$ 0.71	83	-0.16 $\pm$ 0.81	0.31	0.50

148 *SARP1-2 serum IL-6 or SARP3 plasma IL-6; †Kruskal-Wallis test; ‡Generalized linear model adjusted for age, sex, and BMI.

149 Table E7. Correlation of FeNO levels with asthma phenotypes in SARP1-3.

	FeNO Low (<25 ppb)		FeNO High (≥25 ppb)		P value*	P value† (Adjusted)
	n	Mean ± SD	n	Mean ± SD		
Age at enrollment, years	442	45 ± 14	409	42 ± 15	0.017	NA
Gender (% female)	442	70	409	65	0.12	NA
Race (% White/African American/others)	442	66/27/7	409	60/31/9	0.18	NA
Body mass index (BMI)	442	33 ± 8.9	409	31 ± 7.9	0.0008	NA
Baseline FEV ₁ % predicted	442	76 ± 21	409	73 ± 20	0.036	0.0015
Baseline FEV ₁ /FVC	442	0.71 ± 0.11	409	0.68 ± 0.11	<0.0001	<0.0001
Maximal FEV ₁ % predicted	440	86 ± 19	409	86 ± 19	0.66	0.42
Change in % predicted FEV ₁ to albuterol	440	15 ± 16	409	21 ± 19	<0.0001	<0.0001
% Sputum neutrophils	327	51 ± 27	295	46 ± 26	0.01	0.24
% Sputum eosinophils	326	1.9 ± 4.3	294	7.0 ± 12	<0.0001	<0.0001
Blood neutrophil counts (cells/μl)	435	4389 ± 2174	402	4283 ± 2148	0.3	0.68
Blood eosinophil counts (cells/μl)	434	211 ± 187	399	349 ± 302	<0.0001	<0.0001
FeNO (ppb)	442	14 ± 5.4	409	55 ± 37.5	<0.0001	<0.0001
Total serum IgE (IU/ml)	422	289 ± 684	396	381 ± 594	<0.0001	<0.0001
ATS classification (% severe asthma)	442	48	409	43	0.1	0.55
ER or urgent care visit for asthma in last year (% yes)	441	41	409	38	0.53	0.81
Systemic corticosteroid use in last year (% yes)	442	35	409	38	0.32	0.025
Hospitalization for breathing problem in last year (% yes)	441	8	409	11	0.15	0.049
ICU admission for asthma in last year (% yes)	442	1	409	4	0.029	0.024
Serum IL-6 levels (pg/ml) in SARP1-2	157	2.8 ± 4.8	170	2.8 ± 7.2	0.96	0.4
Plasma IL-6 levels (pg/ml) in SARP3	215	2.5 ± 2.2	169	2.6 ± 2.3	0.82	0.059
Serum sIL-6R levels (ng/ml)	392	39 ± 11	358	40 ± 13	0.47	0.09
IL6R rs2228145 (% AA/CA/CC)	412	48/40/13	378	47/38/16	0.54	0.45
% ΔFEV ₁ (corticosteroid evoked) in SARP3	251	2.8 ± 13	204	8.5 ± 19	0.0006	0.0004
ΔACQ6 (corticosteroid evoked) in SARP3	259	-0.13 ± 0.70	214	-0.31 ± 0.82	0.042	0.013

150 *Kruskal-Wallis test; †Generalized linear model adjusted for age, sex, BMI, and race.

151 Table E8. Correlation of blood eosinophil counts with asthma phenotypes in SARP1-3.

	Blood Eosinophil Low (<300 cells/ μ l)		Blood Eosinophil High ($\geq$ 330 cells/ μ l)		P value [†]	P value [‡] (Adjusted)
	n	Mean $\pm$ SD	n	Mean $\pm$ SD		
Age at enrollment, years	547	43 $\pm$ 14	330	44 $\pm$ 15	0.37	NA
Gender (% female)	547	70	330	64	0.06	NA
Race (% White/African American/others)	547	61/31/8	330	63/28/9	0.67	NA
Body mass index (BMI)	547	32 $\pm$ 8.9	329	31 $\pm$ 7.8	0.11	NA
Baseline FEV ₁ % predicted	547	77 $\pm$ 20	329	71 $\pm$ 19	<0.0001	<0.0001
Baseline FEV ₁ /FVC	547	0.72 $\pm$ 0.11	329	0.68 $\pm$ 0.11	<0.0001	<0.0001
Maximal FEV ₁ % predicted	545	87 $\pm$ 19	329	84 $\pm$ 19	0.017	0.024
Change in % predicted FEV ₁ to albuterol	545	16 $\pm$ 16	329	21 $\pm$ 19	<0.0001	<0.0001
% Sputum neutrophils	375	50 $\pm$ 27	250	47 $\pm$ 26	0.16	0.44
% Sputum eosinophils	374	1.8 $\pm$ 4.4	249	8.2 $\pm$ 13	<0.0001	<0.0001
Blood neutrophil counts (cells/ μ l)	547	4257 $\pm$ 2274	330	4406 $\pm$ 1880	0.02	0.005
Blood eosinophil counts (cells/ μ l)	547	140 $\pm$ 73	330	504 $\pm$ 291	<0.0001	<0.0001
FeNO (ppb)	522	28 $\pm$ 27	309	45 $\pm$ 40	<0.0001	<0.0001
Total serum IgE (IU/ml)	524	258 $\pm$ 424	326	487 $\pm$ 1048	<0.0001	<0.0001
ATS classification (% severe asthma)	547	45	330	46	0.73	0.81
ER or urgent care visit for asthma in last year (% yes)	546	40	330	40	0.94	0.62
Systemic corticosteroid use in last year (% yes)	547	33	329	40	0.041	0.027
Hospitalization for breathing problem in last year (% yes)	546	9	329	10	0.72	0.46
ICU admission for asthma in last year (% yes)	547	3	329	3	1	0.99
Serum or Plasma IL-6 levels (pg/ml)*	457	2.8 $\pm$ 5.3	281	2.4 $\pm$ 2.3	0.29	0.17
sIL-6R levels (ng/ml)	481	39 $\pm$ 11	295	40 $\pm$ 12	0.8	0.7
IL6R rs2228145 (% AA/CA/CC)	502	48/38/14	307	47/39/14	0.92	0.92
% Δ FEV ₁ (corticosteroid evoked) in SARP3	292	3.1 $\pm$ 13	166	9.2 $\pm$ 20	0.004	0.0004
Δ ACQ6 (corticosteroid evoked) in SARP3	305	-0.16 $\pm$ 0.76	171	-0.28 $\pm$ 0.80	0.026	0.057

152 *SARP1-2 serum IL-6 or SARP3 plasma IL-6; [†]Kruskal-Wallis test; [‡]Generalized linear model adjusted for age, sex, BMI, and race.

153 Table E9. Correlation of IL-6 and FeNO levels with asthma phenotypes in SARP1-3 (complete table).

	LL (n=278)*		LH (n=249)*		HL (n=88)*		HH (n=90)*		Four Groups	
	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Age at enrollment, years	278	43 $\pm$ 14	249	41 $\pm$ 15	88	47 $\pm$ 13	90	44 $\pm$ 14	0.0017	NA
Gender (% female)	278	67	249	63	88	78	90	72	0.056	NA
Race (% White/African American/others)	278	65/29/6	249	62/29/9	88	68/21/11	90	57/38/5	0.16	NA
Body mass index (BMI)	278	31 $\pm$ 6.9	249	29 $\pm$ 6.9	88	39 $\pm$ 12	90	35 $\pm$ 9.0	<0.0001	NA
Baseline FEV ₁ % predicted	278	77 $\pm$ 21	249	76 $\pm$ 19	88	70 $\pm$ 21	90	70 $\pm$ 19	0.001	0.014
Baseline FEV ₁ /FVC	278	0.71 $\pm$ 0.11	249	0.69 $\pm$ 0.10	88	0.71 $\pm$ 0.12	90	0.69 $\pm$ 0.11	0.0079	0.02
Maximal FEV ₁ % predicted	278	87 $\pm$ 19	249	89 $\pm$ 18	87	80 $\pm$ 18	90	83 $\pm$ 19	0.0003	0.13
Change in % predicted FEV ₁ to albuterol	278	15 $\pm$ 15	249	20 $\pm$ 20	87	17 $\pm$ 19	90	22 $\pm$ 19	<0.0001	0.004
% Sputum neutrophils	203	50 $\pm$ 26	181	44 $\pm$ 27	69	52 $\pm$ 29	62	50 $\pm$ 24	0.08	0.4
% Sputum eosinophils	203	1.5 $\pm$ 3.9	180	7.4 $\pm$ 13	68	2.9 $\pm$ 5.5	62	4.9 $\pm$ 8.1	<0.0001	<0.0001
Blood neutrophil counts (cells/ μ l)	273	3974 $\pm$ 1625	247	3822 $\pm$ 1562	86	5576 $\pm$ 2407	87	5379 $\pm$ 3133	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	273	201 $\pm$ 158	245	339 $\pm$ 286	85	223 $\pm$ 262	86	345 $\pm$ 270	<0.0001	<0.0001
FeNO (ppb)	278	14 $\pm$ 5.4	249	58 $\pm$ 41	88	15 $\pm$ 5.7	90	49 $\pm$ 35	<0.0001	<0.0001
Total serum IgE (IU/ml)	265	282 $\pm$ 730	242	363 $\pm$ 612	84	298 $\pm$ 488	87	437 $\pm$ 614	<0.0001	<0.0001
ATS classification (% severe asthma)	278	42	249	35	88	60	90	44	0.0005	0.24
ER or urgent care visit for asthma in last year (% yes)	277	37	249	33	88	56	90	41	0.0024	0.27
Systemic corticosteroid use in last year (% yes)	278	28	249	27	88	45	90	50	<0.0001	0.0048
Hospitalization for breathing problem in last year (% yes)	277	5	249	10	88	17	90	13	0.003	0.023
ICU admission for asthma in last year (% yes)	278	0	249	4	88	2	90	6	0.013	0.09
Serum or Plasma IL-6 levels (pg/ml)**	278	1.4 $\pm$ 0.69	249	1.3 $\pm$ 0.73	88	6.6 $\pm$ 5.5	90	6.5 $\pm$ 9.3	<0.0001	<0.0001
Serum sIL-6R levels (ng/ml)	238	37 $\pm$ 10	218	39 $\pm$ 13	78	43 $\pm$ 12	70	41 $\pm$ 12	0.0071	0.058
% Δ FEV ₁ (corticosteroid evoked) in SARP3	145	2.6 $\pm$ 13	107	6.8 $\pm$ 16	48	1.4 $\pm$ 15	40	8.8 $\pm$ 18	0.0007	0.039
Δ ACQ6 (corticosteroid evoked) in SARP3	149	-0.13 $\pm$ 0.69	111	-0.25 $\pm$ 0.74	48	-0.19 $\pm$ 0.74	45	-0.43 $\pm$ 0.79	0.17	0.14

154 *LL: low IL-6 and low FeNO, LH: low IL-6 and high FeNO, HL: high IL-6 and low FeNO, HH: high IL-6 and high FeNO; †Kruskal-
155 Wallis test; ‡Generalized linear model adjusted for age, sex, BMI, and race; ** SARP1-2 serum IL-6 or SARP3 plasma IL-6.

156 Table E10. Correlation of IL-6 and FeNO levels with asthma phenotypes in SARP1-2.

	LL (117)*		LH (130)*		HL (35)*		HH (40)*		Four Groups	
	N	mean $\pm$ SD	N	mean $\pm$ SD	N	mean $\pm$ SD	N	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Age at enrollment, years	117	37 $\pm$ 13	130	37 $\pm$ 14	35	43 $\pm$ 13	40	39 $\pm$ 14	0.16	NA
Gender (% female)	117	75	130	65	35	83	40	63	0.08	NA
Race (% Caucasian/African American/others)	117	61/38/2	130	57/38/5	35	74/26/0	40	63/33/5	0.35	NA
Body mass index (BMI)	117	30 $\pm$ 7.3	130	29 $\pm$ 6.9	35	37 $\pm$ 13	40	34 $\pm$ 9.7	0.0003	NA
Baseline FEV ₁ % predicted	117	83 $\pm$ 18	130	75 $\pm$ 20	35	76 $\pm$ 21	40	72 $\pm$ 19	0.0045	0.013
Baseline FEV ₁ /FVC	117	0.75 $\pm$ 0.1	130	0.70 $\pm$ 0.11	35	0.75 $\pm$ 0.12	40	0.72 $\pm$ 0.11	0.0003	0.0084
Maximal FEV ₁ % predicted	117	92 $\pm$ 15	130	89 $\pm$ 17	35	83 $\pm$ 18	40	87 $\pm$ 18	0.07	0.22
Change in % predicted FEV ₁	117	13 $\pm$ 14	130	21 $\pm$ 21	35	12 $\pm$ 17	40	21 $\pm$ 16	<0.0001	0.0033
% Sputum neutrophils	77	41 $\pm$ 26	93	37 $\pm$ 27	28	43 $\pm$ 29	27	42 $\pm$ 20	0.5	0.6
% Sputum eosinophils	77	1.3 $\pm$ 2.8	92	6.1 $\pm$ 11.1	27	4.1 $\pm$ 6.7	27	5.9 $\pm$ 9.2	<0.0001	<0.0001
Blood neutrophil counts (cells/ μ l)	112	3947 $\pm$ 1434	128	3688 $\pm$ 1345	33	5370 $\pm$ 2420	37	5068 $\pm$ 3561	<0.0001	0.0047
Blood eosinophil counts (cells/ μ l)	112	180 $\pm$ 137	126	300 $\pm$ 177	32	225 $\pm$ 359	36	275 $\pm$ 221	<0.0001	0.0001
FeNO (ppb)	117	14 $\pm$ 5.6	130	63 $\pm$ 45	35	14 $\pm$ 6.4	40	55 $\pm$ 36	<0.0001	<0.0001
Total serum IgE (IU/ml)	107	331 $\pm$ 1046	124	336 $\pm$ 456	32	148 $\pm$ 307	37	346 $\pm$ 545	<0.0001	0.042
ATS classification (% severe asthma)	117	21	130	22	35	34	40	23	0.41	0.9
ER or urgent care visit for asthma in last year (% yes)	116	33	130	30	35	54	40	35	0.06	0.26
Systemic corticosteroid use (% yes)	117	3	130	5	35	6	40	13	0.12	0.22
Hospitalization for breathing problem (% yes)	116	1	130	9	35	6	40	10	0.028	0.09
ICU admission for asthma in last year (% yes)	117	0	130	3	35	0	40	5	0.11	0.92
Serum IL-6 levels (pg/ml)	117	1.3 $\pm$ 0.74	130	1.3 $\pm$ 0.73	35	8.0 $\pm$ 8.2	40	7.9 $\pm$ 14	<0.0001	<0.0001
Serum sIL-6R levels (ng/ml)	117	37 $\pm$ 11	129	40 $\pm$ 16	35	43 $\pm$ 15	40	41 $\pm$ 13	0.19	0.31

157 *LL: low IL-6 and low FeNO, LH: low IL-6 and high FeNO, HL: high IL-6 and low FeNO, HH: high IL-6 and high FeNO; †Kruskal-

158 Wallis test; ‡Generalized linear model adjusted for age, sex, BMI, and race.

159 Table E11. Correlation of IL-6 and FeNO levels with asthma phenotypes in SARP3.

	LL (161)*		LH (119)*		HL (53)*		HH (50)*		Four Groups	
	N	mean $\pm$ SD	N	mean $\pm$ SD	N	mean $\pm$ SD	N	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Age at enrollment, years	161	48 $\pm$ 13	119	45 $\pm$ 14	53	51 $\pm$ 11	50	48 $\pm$ 14	0.08	NA
Gender (% female)	161	61	119	61	53	75	50	80	0.029	NA
Race (% Caucasian/African American/others)	161	68/22/9	119	68/19/13	53	64/17/19	50	52/42/6	0.015	NA
Body mass index (BMI)	161	31 $\pm$ 6.5	119	29 $\pm$ 6.9	53	40 $\pm$ 10	50	35 $\pm$ 8.5	<0.0001	NA
Baseline FEV ₁ % predicted	161	73 $\pm$ 22	119	76 $\pm$ 19	53	66 $\pm$ 19	50	67 $\pm$ 19	0.0036	0.09
Baseline FEV ₁ /FVC	161	0.68 $\pm$ 0.12	119	0.68 $\pm$ 0.10	53	0.69 $\pm$ 0.12	50	0.67 $\pm$ 0.1	0.42	0.47
Maximal FEV ₁ % predicted	161	83 $\pm$ 20	119	89 $\pm$ 18	52	78 $\pm$ 18	50	81 $\pm$ 20	0.0018	0.16
Change in % predicted FEV ₁	161	17 $\pm$ 16	119	19 $\pm$ 18	52	21 $\pm$ 19	50	23 $\pm$ 21	0.08	0.14
% Sputum neutrophils	126	55 $\pm$ 25	88	52 $\pm$ 25	41	59 $\pm$ 2	35	57 $\pm$ 25	0.52	0.68
% Sputum eosinophils	126	1.6 $\pm$ 4.4	88	8.7 $\pm$ 14	41	2.1 $\pm$ 4.4	35	4.1 $\pm$ 7.2	<0.0001	<0.0001
Blood neutrophil counts (cells/ μ l)	161	3993 $\pm$ 1750	119	39671 $\pm$ 1761	53	5704 $\pm$ 2412	50	5610 $\pm$ 2791	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	161	215 $\pm$ 170	119	379 $\pm$ 364	53	221 $\pm$ 185	50	395 $\pm$ 293	<0.0001	<0.0001
FeNO (ppb)	161	14 $\pm$ 5.3	119	54 $\pm$ 36	53	15 $\pm$ 5.2	50	44 $\pm$ 34	<0.0001	<0.0001
Total serum IgE (IU/ml)	158	248 $\pm$ 393	118	391 $\pm$ 742	52	389 $\pm$ 555	50	504 $\pm$ 658	0.0002	0.0003
ATS classification (% severe asthma)	161	57	119	49	53	77	50	62	0.0053	0.22
ER or urgent care visit for asthma in last year (% yes)	161	40	119	37	53	57	50	46	0.09	0.69
Systemic corticosteroid use (% yes)	161	47	119	50	53	72	50	80	<0.0001	0.0021
Hospitalization for breathing problem (% yes)	161	8	119	10	53	25	50	16	0.01	0.08
ICU admission for asthma in last year (% yes)	161	1	119	5	53	4	50	6	0.1	0.25
Plasma IL-6 levels (pg/ml)	161	1.5 $\pm$ 0.65	119	1.4 $\pm$ 0.72	53	5.7 $\pm$ 2.3	50	5.4 $\pm$ 2.4	<0.0001	<0.0001
Serum sIL-6R levels (ng/ml)	121	38 $\pm$ 9.3	89	39 $\pm$ 9.5	43	42 $\pm$ 9.3	30	40 $\pm$ 9.4	0.043	0.29
% Δ FEV ₁ (corticosteroid evoked) in SARP3	145	2.6 $\pm$ 13	107	6.8 $\pm$ 16	48	1.4 $\pm$ 15	40	8.8 $\pm$ 18	0.0007	0.039
Δ ACQ6 (corticosteroid evoked) in SARP3	149	-0.13 $\pm$ 0.69	111	-0.25 $\pm$ 0.74	48	-0.19 $\pm$ 0.74	45	-0.43 $\pm$ 0.79	0.17	0.14

160 *LL: low IL-6 and low FeNO, LH: low IL-6 and high FeNO, HL: high IL-6 and low FeNO, HH: high IL-6 and high FeNO; †Kruskal-

161 Wallis test; ‡Generalized linear model adjusted for age, sex, BMI, and race.

162 Table E12. Correlation of IL-6 levels and blood eosinophil count with asthma phenotypes in SARP1-3 (complete table).

	LL (344)*		LH (216)*		HL (113)*		HH (65)*		Four Groups	
	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Age at enrollment, years	344	41 $\pm$ 14	216	42 $\pm$ 15	113	45 $\pm$ 13	65	47 $\pm$ 15	0.013	NA
Gender (% female)	344	67	216	63	113	80	65	65	0.016	NA
Race (% White/African American/others)	344	63/30/7	216	63/29/8	113	57/33/10	65	68/26/6	0.88	NA
Body mass index (BMI)	344	30 $\pm$ 7.0	215	30 $\pm$ 7.1	113	38 $\pm$ 11	65	35 $\pm$ 9.3	<0.0001	NA
Baseline FEV ₁ % predicted	344	78 $\pm$ 20	215	73 $\pm$ 19	113	71 $\pm$ 19	65	68 $\pm$ 20	<0.0001	0.0033
Baseline FEV ₁ /FVC	344	0.71 $\pm$ 0.11	215	0.68 $\pm$ 0.10	113	0.72 $\pm$ 0.11	65	0.68 $\pm$ 0.12	0.0003	0.01
Maximal FEV ₁ % predicted	344	89 $\pm$ 19	215	86 $\pm$ 19	112	82 $\pm$ 17	65	81 $\pm$ 21	0.0002	0.12
Change in % predicted FEV ₁ to albuterol	344	15 $\pm$ 16	215	20 $\pm$ 18	112	18 $\pm$ 18	65	22 $\pm$ 21	<0.0001	0.004
% Sputum neutrophils	229	50 $\pm$ 27	165	45 $\pm$ 26	79	52 $\pm$ 27	51	52 $\pm$ 26	0.17	0.58
% Sputum eosinophils	229	1.7 $\pm$ 4.1	164	8.1 $\pm$ 13	78	2.7 $\pm$ 5.8	51	5.6 $\pm$ 8.1	<0.0001	<0.0001
Blood neutrophil counts (cells/ μ l)	344	3818 $\pm$ 1694	216	4003 $\pm$ 1459	113	5320 $\pm$ 2800	65	5578 $\pm$ 2631	<0.0001	<0.0001
Blood eosinophil counts (cells/ μ l)	344	136 $\pm$ 70	216	480 $\pm$ 262	113	141 $\pm$ 80	65	524 $\pm$ 305	<0.0001	<0.0001
FeNO (ppb)	327	28 $\pm$ 29	197	47 $\pm$ 44	108	27 $\pm$ 23	63	41 $\pm$ 38	<0.0001	<0.0001
Total serum IgE (IU/ml)	330	237 $\pm$ 405	214	508 $\pm$ 1176	108	329 $\pm$ 492	65	399 $\pm$ 619	<0.0001	<0.0001
ATS classification (% severe asthma)	344	37	216	40	113	57	65	43	0.0026	0.14
ER or urgent care visit for asthma in last year (% yes)	343	36	216	37	113	50	65	45	0.028	0.65
Systemic corticosteroid use in last year (% yes)	344	25	215	29	113	44	65	54	<0.0001	0.002
Hospitalization for breathing problem in last year (% yes)	343	8	215	7	113	14	65	18	0.007	0.1
ICU admission for asthma in last year (% yes)	344	2	215	3	113	5	65	3	0.47	0.65
Serum or Plasma IL-6 levels (pg/ml)**	344	1.3 $\pm$ 0.71	216	1.5 $\pm$ 0.74	113	7.2 $\pm$ 9.3	65	5.5 $\pm$ 3.1	<0.0001	<0.0001
Serum sIL-6R levels (ng/ml)	294	38 $\pm$ 11	195	39 $\pm$ 13	97	42 $\pm$ 11	51	40 $\pm$ 13	0.005	0.70
% Δ FEV ₁ (corticosteroid evoked) in SARP3	164	3.3 $\pm$ 13	92	6.2 $\pm$ 17	55	0.4 $\pm$ 11	33	12 $\pm$ 21	0.008	0.004
Δ ACQ6 (corticosteroid evoked) in SARP3	170	-0.14 $\pm$ 0.71	94	-0.23 $\pm$ 0.79	58	-0.24 $\pm$ 0.83	35	-0.42 $\pm$ 0.64	0.07	0.16

163 *LL: low IL-6 and low blood eosinophil count, LH: low IL-6 and high blood eosinophil count, HL: high IL-6 and low blood
164 eosinophil count, HH: high IL-6 and high blood eosinophil count; †Kruskal-Wallis test; ‡Generalized linear model adjusted for age,
165 sex, BMI, and race; ** SARP1-2 serum IL-6 or SARP3 plasma IL-6.

166 Table E13. Correlation of FeNO levels and blood eosinophil count with asthma phenotypes in SARP1-3.

	LL (n=327)*		LH (n=106)*		HL (n=195)*		HH (n=203)*		Four Groups	
	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	n	mean $\pm$ SD	P value†	P value‡ (Adjusted)
Age at enrollment, years	327	44 $\pm$ 14	106	45 $\pm$ 14	195	41 $\pm$ 14	203	43 $\pm$ 15	0.027	NA
Gender (% female)	327	73	106	60	195	64	203	67	0.033	NA
Race (% White/African American/others)	327	65/27/8	106	69/24/7	195	56/35/9	203	63/27/10	0.34	NA
Body mass index (BMI)	327	33 $\pm$ 9.3	106	32 $\pm$ 7.7	195	31 $\pm$ 8.0	203	31 $\pm$ 7.9	0.013	NA
Baseline FEV ₁ % predicted	327	77 $\pm$ 21	106	72 $\pm$ 20	195	77 $\pm$ 19	203	70 $\pm$ 19	<0.0001	<0.0001
Baseline FEV ₁ /FVC	327	0.72 $\pm$ 0.11	106	0.69 $\pm$ 0.12	195	0.71 $\pm$ 0.10	203	0.67 $\pm$ 0.10	<0.0001	<0.0001
Maximal FEV ₁ % predicted	325	87 $\pm$ 19	106	83 $\pm$ 20	195	89 $\pm$ 18	203	85 $\pm$ 19	0.046	0.14
Change in % predicted FEV ₁ to albuterol	325	15 $\pm$ 16	106	16 $\pm$ 14	195	17 $\pm$ 16	203	24 $\pm$ 21	<0.0001	<0.0001
% Sputum neutrophils	237	51 $\pm$ 27	85	53 $\pm$ 24	134	48 $\pm$ 26	155	44 $\pm$ 27	0.021	0.06
% Sputum eosinophils	236	1.3 $\pm$ 3.1	85	3.6 $\pm$ 6.5	134	2.8 $\pm$ 6.1	154	10.8 $\pm$ 14.8	<0.0001	<0.0001
Blood neutrophil counts (cells/ μ l)	327	4299 $\pm$ 2160	106	4599 $\pm$ 2087	195	4227 $\pm$ 2484	203	4317 $\pm$ 1758	0.045	0.08
Blood eosinophil counts (cells/ μ l)	327	132 $\pm$ 78	106	454 $\pm$ 223	195	154 $\pm$ 75	203	535 $\pm$ 319	<0.0001	<0.0001
FeNO (ppb)	327	14 $\pm$ 5.4	106	15 $\pm$ 5.2	195	50 $\pm$ 32	203	60 $\pm$ 42	<0.0001	<0.0001
Total serum IgE (IU/ml)	312	222 $\pm$ 408	105	496 $\pm$ 1157	188	327 $\pm$ 458	200	430 $\pm$ 695	<0.0001	<0.0001
ATS classification (% severe asthma)	327	49	106	49	195	38	203	46	0.1	0.46
ER or urgent care visit for asthma in last year (% yes)	326	40	106	41	195	38	203	38	0.94	0.95
Systemic corticosteroid use in last year (% yes)	327	33	106	41	195	35	203	41	0.2	0.051
Hospitalization for breathing problem in last year (% yes)	326	9	106	6	195	9	203	13	0.18	0.11
ICU admission for asthma in last year (% yes)	327	2	106	0	195	4	203	4	0.08	0.24
Serum or Plasma IL-6 levels (pg/ml)**	276	2.7 $\pm$ 3.9	88	2.2 $\pm$ 1.8	159	2.8 $\pm$ 7.2	172	2.6 $\pm$ 2.7	0.29	0.19
Serum sIL-6R levels (ng/ml)	290	39 $\pm$ 11	93	38 $\pm$ 11	166	39 $\pm$ 12	181	41 $\pm$ 13	0.53	0.2
% Δ FEV ₁ (corticosteroid evoked) in SARP3	185	1.2 $\pm$ 11	66	7.1 $\pm$ 16	106	6.4 $\pm$ 14	98	10.9 $\pm$ 22	0.0004	<0.0001
Δ ACQ6 (corticosteroid evoked) in SARP3	192	-0.14 $\pm$ 0.7	67	-0.10 $\pm$ 0.69	111	-0.19 $\pm$ 0.85	102	-0.44 $\pm$ 0.77	0.009	0.005

167 *LL: low FeNO and low blood eosinophil count, LH: low FeNO and high blood eosinophil count, HL: high FeNO and low blood
168 eosinophil, HH: high FeNO and high blood eosinophil count; †Kruskal-Wallis test; ‡Generalized linear model adjusted for age, sex,
169 BMI, and race; ** SARP1-2 serum IL-6 or SARP3 plasma IL-6.

170 Table E14. Correlation of biomarkers.

	% Sputum neutrophils	% Sputum eosinophils	Blood neutrophil count (cells/ μ l)	Blood eosinophil count (cells/ μ l)	FeNO (ppb)	Total serum IgE (IU/ml)	IL-6 levels (pg/ml)
% Sputum neutrophils		-0.07 (0.090)	0.10 (0.013)	-0.03 (0.45)	-0.12 (0.0020)	-0.09 (0.028)	0.077 (0.075)
% Sputum eosinophils	-0.07 (0.090)		-0.0032 (0.94)	0.43 (<0.0001)	0.37 (<0.0001)	0.21 (<0.0001)	0.02 (0.64)
Blood neutrophil count (cells/ μ l)	0.10 (0.013)	-0.0032 (0.94)		0.053 (0.12)	-0.075 (0.031)	-0.05 (0.14)	0.36 (<0.0001)
Blood eosinophil count (cells/ μ l)	-0.03 (0.45)	0.43 (<0.0001)	0.053 (0.12)		0.32 (<0.0001)	0.27 (<0.0001)	0.08 (0.054)
FeNO (ppb)	-0.12 (0.0020)	0.37 (<0.0001)	-0.075 (0.031)	0.32 (<0.0001)		0.27 (<0.0001)	-0.034 (0.37)
Total serum IgE (IU/ml)	-0.09 (0.028)	0.21 (<0.0001)	-0.05 (0.14)	0.27 (<0.0001)	0.27 (<0.0001)		-0.005 (0.89)
IL-6 levels (pg/ml)	0.077 (0.075)	0.02 (0.64)	0.36 (<0.0001)	0.08 (0.054)	-0.034 (0.37)	-0.005 (0.89)	

171 Note: Spearman rank correlation was performed with correlation coefficient rho (p-value) reported. Entries with $|\text{rho}| \geq 0.2$ were labeled
 172 in red color.

Figure Legends

Figure E1. Scatter plot of IL-6 and FeNO levels in SARP1-3 (n=705). Subjects were categorized into high IL-6 (≥ 3.1 pg/ml,) or low IL-6 (< 3.1 pg/ml) [IL-6 levels were logarithm transformed and cutoff value of 3.1 pg/ml was shown in red line], and high FeNO (≥ 25 ppb) or low FeNO (< 25 ppb) [FeNO levels were logarithm transformed and cutoff value of 25 ppb was shown in green line].

Figure E2. Scatter plot of IL-6 levels and blood eosinophil counts in SARP1-3 (n=705). Subjects were categorized into high IL-6 (≥ 3.1 pg/ml,) or low IL-6 (< 3.1 pg/ml) [IL-6 levels were logarithm transformed and cutoff value of 3.1 pg/ml was shown in red line], and high blood eosinophil counts (≥ 300 cells/ μ l) or low blood eosinophil counts (< 300 cells/ μ l) [blood eosinophil counts were logarithm transformed and cutoff value of 300 cells/ μ l was shown in green line].

Figure E3. Summary of asthma endotypes defined by IL-6 and FeNO levels.

