

RESEARCH LETTER

IL-6 pathway upregulation in subgroup of severe asthma is associated with neutrophilia and poor lung function

N. Turan¹  | M. J. Edwards¹ | S. Bates¹ | D. Shaw² | K. F. Chung³ | M. J. Loza⁴ | A. James⁵ | A. Van Oosterhout¹ | the U-BIOPRED Study Group

¹GlaxoSmithKline, Respiratory R&D, Stevenage, UK

²Respiratory Research Unit, University of Nottingham, Nottingham, UK

³National Heart & Lung Institute, Imperial College, London, UK

⁴Janssen Research & Development, Spring House, PA, USA

⁵Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Correspondence

N. Turan, GlaxoSmithKline, Respiratory R&D, Stevenage, UK

Email: nil.c.turan-jurdzinski@gsk.com

Funding Information

UBIOPRED consortium

To the Editor

Interleukin-6 (IL-6) is a pleiotropic cytokine and is a member of the gp130-related cytokine family. It is produced by many different cell types including the myeloid lineage cells, T cells and structural cells such as endothelial cells. The IL-6 receptor consists of a heterodimer formed from a specific IL-6R which has limited distribution with expression in a narrow spectrum of immune cells including neutrophils and T cells, and it associates with gp130, which is expressed ubiquitously on all cells. The formation of the ligand-heterodimeric receptor complex initiates a JAK-STAT3 signalling cascade, which also triggers activation of the MAPK and PI3K pathways.¹ A soluble form of the IL-6R can be produced via either proteolytic cleavage or alternative splicing of mRNA, and the IL-6-sIL-6R complex can associate with gp130 and drive a signalling cascade. Therefore, whilst the limited expression of IL-6R limits the extent of classic signalling of IL-6, the trans-signalling provides a mechanism for cells such as fibroblasts and endothelial cells that only express the gp130 component of the receptor.² IL-6 has extensive pro-inflammatory activities including in the acute phase response following infection with systemic induction of C-reactive protein, complement component C3, fibrinogen and serum amyloid A protein.³ Infection and TLR stimulus also induce a combination of cytokines including IL-6 that drives neutrophil attracting chemokine production from local structural cells.¹ IL-6-primed neutrophils have been shown to be activated being able to increase reactive oxygen species, elastase and PAF production.⁴ In addition, IL-6 plays a significant role in the adaptive response inducing maturation of B cells to produce IgG, IgM and IgA. In T cells, it has been

shown to induce Th2 response with naïve CD4 T cells, whilst it also plays a role in the balance of Th17 and T-regulatory (Treg) cells, being able to suppress Treg induction whilst combining with TGF- β to generate Th17 cells.⁵ IL-17 pathway has been reported to play a role for neutrophil recruitment in severe asthma with frequent exacerbations.⁶

Given the pleiotropic nature of IL-6 and its involvement in many types of inflammation, including Th2 induction in T cells, a possible involvement in asthma has been suggested. This has been supported by increased IL-6 levels in the sputum of severe asthma patients and increased IL-6 and sIL-6R in serum during asthma attacks.⁷ In addition, murine models of asthma have indicated a beneficial effect of blocking of IL-6R, which was shown to alter the balance between the Th2 cell response and the T-regulatory responses with a greater number of Treg cells produced that had immunosuppressive activity.⁵ Furthermore, analysis of GWAS data has identified IL6R SNP as an asthma susceptibility locus.⁸ Hawkins et al. have shown genetic association of polymorphisms in the IL6R gene with severity of asthma, high levels of sIL-6R and poor lung function in the SARP cohort.⁹

As asthma is a heterogeneous disease, we were interested to investigate whether IL-6 pathway upregulation is associated with particular clinical characteristics. Here, we have used a hypothesis-driven analysis by investigating sputum transcriptomics, sputum and blood proteomics and clinical data in a large cohort of severe non-smoker asthma patients from the U-BIOPRED consortium.¹⁰ The non-smoker group is defined as those with less than 5 pack-years of smoking and has not smoked for 1 year prior to recruitment.

Sputum IL6R mRNA levels were significantly different between healthy individuals ($n = 16$) and non-smoking patients with severe asthma ($n = 61$) (fold change 2.78, $FDR < 0.002$). However, IL6R levels were still heterogeneous within the groups (Figure 1A). We used a hierarchical clustering approach starting from genes that are highly correlated with IL6R in the sputum, as these genes are likely to provide biologically relevant connections to the IL-6 pathway. Indeed, pathway analysis using Thomson Reuters MetaCore™ software suite (version 6.24) has shown that these genes were significantly enriched in expected pathways related to immune response signalling including IL-6 signalling, signalling via the JAK-STAT pathway, and inhibition of neutrophil migration in the COPD pathway ($FDR < 0.1$). There were also statistically significant enrichments for metabolic pathways, oxidation-reduction process, MHC class II protein complex assembly and cell death. We then used these genes that are highly correlated with IL6R to group patients by performing a hierarchical clustering approach. The clustering analysis led to two distinct groups (named severe asthma with high IL6R and low IL6R), as shown in Figure 1B. The mean levels of the high-IL6R group were 2.8-fold higher than

those of the low-IL6R group and 3.5-fold higher than healthy levels, whereas the mean of the low-IL6R group was similar to that of the healthy individuals (FC 1.2), as shown in Figure 1C. The high-IL6R group had significantly ($P < .001$) higher neutrophil percentages in the sputum (76%) compared with low-IL6R patients (42%) and healthy individuals (42%) (Figure 1D and Table 1). Stratifying patients using the clinical cut-off of high neutrophilia (sputum neutrophils $\geq 75\%$) or low neutrophilia ($< 75\%$) has led to statistically significant different proportion in the high- and low-IL6R groups (Table 1). These patients had worse lung function post-bronchodilator (Table 1). The high-IL6R group also had significantly higher levels of IL-6 protein in the sputum and higher levels of serum hCRP and IL-6 (*MSD data*) (Table 1). Additional analytes from serum including IL-6R (Table 1), IL-1 α and LPS-binding protein, and other analytes (data not shown) measured as described in methods (provided in the supporting document), did not significantly differ between the high-IL-6R and low-IL-6R groups at a threshold of $P < .05$ and $FC > 1.5$.

There was no significant difference in percentages of sputum and blood eosinophils, ACQ, FENO, BMI, sex, smoking history, atopy

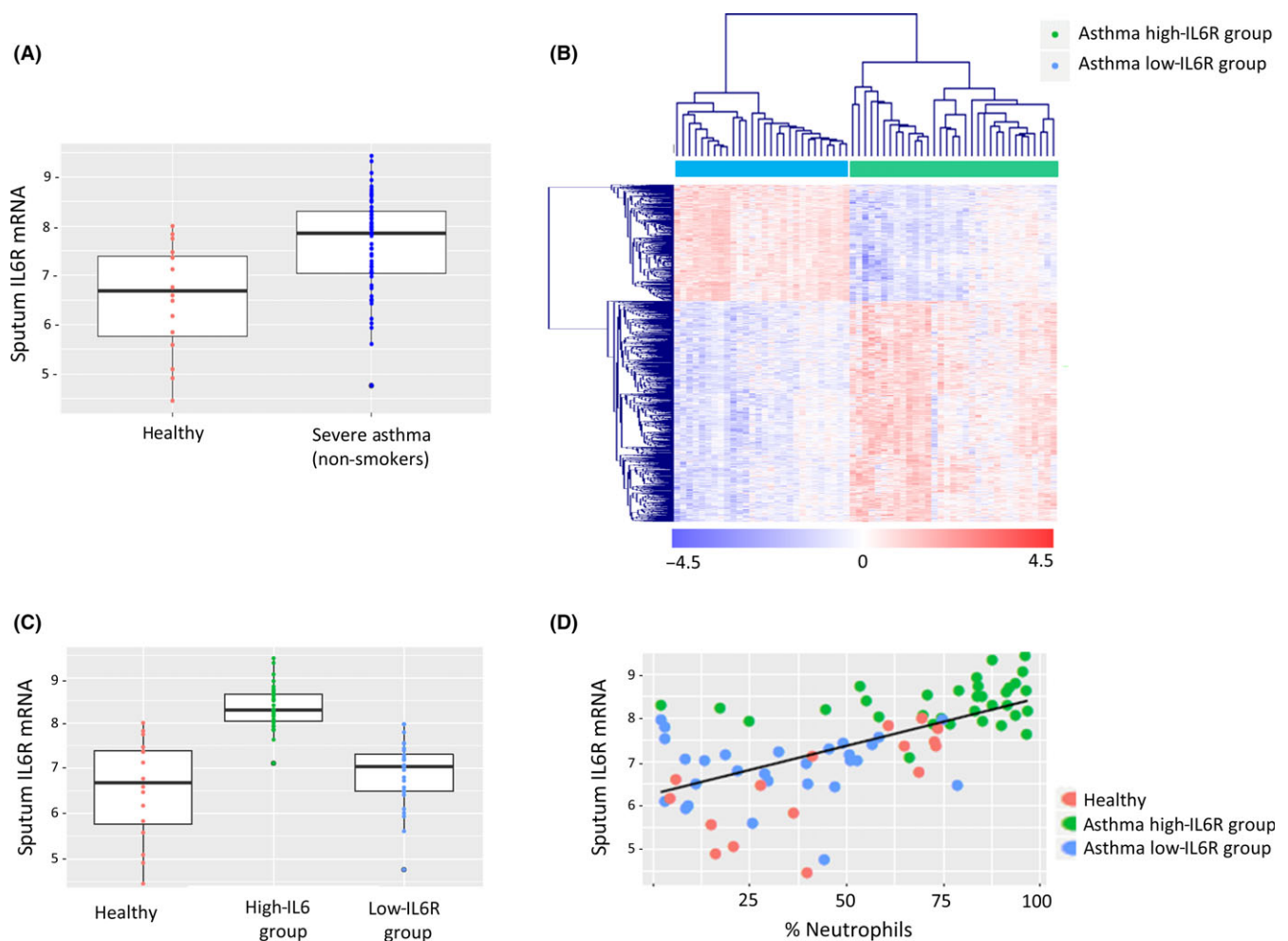


FIGURE 1 A, Levels of IL6R mRNA in the sputum of patients with severe asthma and healthy controls. B, Heatmap showing hierarchical clustering of patients with severe asthma (columns) and genes correlated with IL6R (rows). C, Sputum IL6R mRNA levels in healthy controls and high- and low-IL6R patients with severe asthma. D, IL6R levels and neutrophil percentages

TABLE 1 Patient characteristics, lung function, sputum and blood cell counts, and selected variables from IL6 pathway from transcriptomics and proteomics levels between the high- and low-IL6R groups

	Asthma high IL6R	Asthma low IL6R	Healthy	P-value (High vs Low)
# of patients	33	28	16	
Demographics				
Age at enrolment (y)	55.5 (11)	52 (13.5)	38.1 (13)	.28
Duration of disease (y)	31.7	27.2	-	.28
BMI (kg/m ²)	28.01 (5.28)	27.76 (5.33)	25.78 (2.79)	.85
Female (%)	61% (n = 20)	61% (n = 17)	25% (n = 4)	1
Medication				
Subjects taking OCS %	42% (n = 14)	39% (n = 11)	-	1
OCS dose	10 (5)	11.95 (6.93)	-	.48
Smoking history				
Ex-smokers	21% (n = 7)	26% (n = 7)	6%	.76
Smoking stop (y)	19.14	19.57	8	.95
Cigarettes average	4.57	3.12	5	.4
Smoking (y)	16.71	15	4	.78
Atopy (positive)	66.67% (n = 22)	75% 4/13 (n = 21)	25% (n = 4)	.75
Lung function (pre-bronchodilator)				
FEV ₁ (L)	1.67 (0.86)	1.78 (0.75)	-	.60
FEV ₁ % predicted	56.02 (22.41)	62.24 (17.32)	-	.23
FVC % predicted	83.32 (17.38)	90.24 (16.09)	-	.112
FEV ₁ /FVC	54.03 (14.64)	57.14 (10.73)	-	.344
Lung function (post-bronchodilator)				
FEV ₁ % predicted	62.49 (23.74)	73.81 (17.80)	-	.038
FVC % predicted	91.15 (20.22)	101.15 (14.59)	-	.029
FEV ₁ reversibility (%)	12.73 (10.56)	21.65 (21.88)	-	.06
FENO (p.p.b.)	35.26	47.44	19.71	.26
ACQ7	2.68	2.62	-	.87
Sputum induction				
Total inflammatory cell count (no)	522 (47.46)	531.03 (58.04)	525.13 (43.39)	.51
Neutrophils %	77.04 (19.96)	40.12 (21.71)	43.16 (25.85)	6.22E-09
Eosinophils % (GM)	2.35	2.83	0	.31
Neutrophilic or mix (>75%)	61% (n = 20)	0.04% (n = 1)	0%	1.655e-06
Blood				
Neutrophils %	63.77 (11)	57.07 (11.86)	61.02 (8.21)	.03
Eosinophils % (GM)	3.40	4.12	2.00	.45
IL6 MSD (log2)	0.35 (1.17)	-0.59 (1.04)	-1.15 (0.86)	.002
CRP (log2)	2.11 (2.22)	0.47 (1.72)	-0.55 (1.11)	.002
IL6R Luminex (log2)	13.37 (0.37)	13.41 (0.26)	13.36 (0.17)	.68
Sputum transcriptomics (log2)				
IL6R (226333_at)	8.35 (0.49)	6.86 (0.73)	6.55 (1.11)	2.82E-11
Sputum proteomics (log2)				
Sputum IL6 protein	N = 15	N = 22	N = 13	
	9.59	8.44	8.50	.015

Healthy levels are also reported as information. Mean levels and standard deviation of the groups are reported for all variables except for percentage of eosinophils, which is reported as median.

and medication between the two groups (Table 1). No significant differences in IL6 blood levels were identified between the high-IL-6R group with high ($\geq 3\%$) and low ($< 3\%$) sputum eosinophils

(P-value .78), which supports the independent role of eosinophils in the high-IL6R subgroup. In line with these observations, Peters et al. recently reported a subpopulation of asthma patients with high IL6

and CRP levels in blood associated with asthma severity and blood neutrophils but not blood eosinophil percentages.¹¹ However, sputum neutrophil percentages and sputum IL6 mRNA expression levels were not significantly different between the high- and low-IL6 subgroups in that study.¹¹ These results of higher systemic levels of IL-6 and CRP being associated with the high-IL6 subgroup are consistent with those in our study; however, in contrast we also observed that these patients have neutrophilic airways, as assessed in sputum samples.

We have identified a group of patients with high IL6R mRNA level and high IL-6 protein sputum levels associated with higher sputum neutrophils regardless of eosinophilic inflammation status. This group has also poor lung function and higher levels of systemic IL-6 and CRP. This subpopulation of patients with severe asthma currently has a high unmet clinical need, and they could potentially benefit from therapy targeting the IL-6 pathway.

ACKNOWLEDGEMENTS

This paper is presented on behalf of the U-BIOPRED Study Group with input from the U-BIOPRED Patient Input Platform, Ethics Board and Safety Management Board. We thank all the members of each recruiting centre for their dedicated effort, devotion, promptness and care in the recruitment and assessment of the participants in this study. We would also like to thank Bakke PS, Caruso M, Chanez P, Dahlen SE, Horvath I, Krug N, Montuschi P, Sanak M, Sandstrom T, Djukanovic R, Singer F, Fleming LJ, Fowler SJ and Bansal AT for their contributions to the study.

ORCID

N. Turan  <http://orcid.org/0000-0002-6841-4395>

REFERENCES

- Schaper F, Rose-John S. Interleukin-6: biology, signaling and strategies of blockade. *Cytokine Growth Factor Rev*. 2015;26:475-487.
- Garbers C, Hermanns HM, Schaper F, Muller-Newen G, Grotzinger J, Rose-John S, et al. Plasticity and cross-talk of interleukin 6-type cytokines. *Cytokine Growth Factor Rev*. 2012;23:85-97.

- Baumann H, Gauldie J. The acute phase response. *Immunol Today*. 1994;15:74-80.
- Johnson JL, Moore EE, Tamura DY, Zallen G, Biffl WL, Silliman CC. Interleukin-6 augments neutrophil cytotoxic potential via selective enhancement of elastase release. *J Surg Res*. 1998;76:91-94.
- Rath T, Billmeier U, Waldner MJ, Atreya R, Neurath MF. From physiology to disease and targeted therapy: interleukin-6 in inflammation and inflammation-associated carcinogenesis. *Arch Toxicol*. 2015;89:541-554.
- Ricciardolo FLM, Sorbello V, Folino A, Gallo F, Massaglia GM, Favata G, et al. Identification of IL-17F/frequent exacerbator endotype in asthma. *J Allergy Clin Immunol*. 2017;140:395-406.
- Morjaria JB, Babu KS, Vijayanand P, Chauhan AJ, Davies DE, Holgate ST. Sputum IL-6 concentrations in severe asthma and its relationship with FEV1. *Thorax*. 2011;66:537.
- Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, Le SP, et al. Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet*. 2011;378:1006-1014.
- Hawkins GA, Robinson MB, Hastie AT, Li X, Li H, Moore WC, et al. The IL6R variation Asp(358)Ala is a potential modifier of lung function in subjects with asthma. *J Allergy Clin Immunol*. 2012;130:510-515.
- Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J*. 2015;46:1308-1321.
- Peters MC, McGrath KW, Hawkins GA, Hastie AT, Levy BD, Israel E, et al. Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts. *Lancet Respir Med*. 2016;4:574-584.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Turan N, Edwards MJ, Bates S, et al.; the U-BIOPRED Study Group. IL-6 pathway upregulation in subgroup of severe asthma is associated with neutrophilia and poor lung function. *Clin Exp Allergy*. 2018;48:475-478. <https://doi.org/10.1111/cea.13085>

Online Supplementary Material

IL-6 pathway upregulation in severe asthma is associated with neutrophilia with poor lung function

Nil Turan PhD^{1*}, Matthew J. Edwards PhD¹, Stewart Bates PhD¹, Dominick Shaw MD FRCP², Chung KF MD³, Matthew J Loza PhD⁴, Anna James PhD⁵, Antoon Van Oosterhout PhD^{1*}, and the U-BIOPRED study group

¹GlaxoSmithKline, Respiratory R&D, Stevenage, UK

²Respiratory Research Unit, University of Nottingham, Nottingham, UK

³National Heart & Lung Institute, Imperial College, London, UK

⁴Janssen Research & Development, Spring House, PA, USA

⁵Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

*corresponding author

Material and Methods

Data source from UBIOPRED consortium

In this study, we have used various types of datasets from severe asthma non smoker patients and healthy participants from the Unbiased BIOMarkers in PREdiction of respiratory disease outcomes (Ubiopred) consortium⁽¹⁾. The study design and population has been previously described¹. The datasets that has been used include sputum microarray, sputum and blood somalogic proteomics, inflammatory mediators from blood measured with luminex technology, serum IL6 levels measured with MDS and clinical data.

Microarray analysis of mRNA

Expression profiling was performed using Affymetrix HT HG-U133 + PM GeneChip (Affymetrix, Santa Clara, Calif) and it was described in a previous study². Transcriptomics data was generated with Affymetrix on total RNA extracted from induced sputum samples from severe asthma non smoker patients (n=61) and healthy (n= 16). Only samples with RNA integrity number > 6.5 were used for amplification. Raw data were quality assessed and pre-processed by robust multi-array average normalization using the standard options.

Somalogic proteomics data from sputum

The protocol for somalogic data was described previously². Briefly, analytes were measured by SomaScan v3 platform (SomaLogic, Boulder, Colo; www.somalogic.com) with SOMAmer (Slow Off-rate Modified Aptamer). Analyte levels were reported as relative fluorescence units, cross-plate calibrated, and median normalized.

Luminex biomarkers

Multiplex assays were designed from the R&D Systems Human Luminex Screening Assay (LXSAH) panel of reagents for the following 23 analytes: CCL20, CCL28, CCL13, CD30,

CD40L, C5a, CD26, Galectin-3, GM-CSF, IL-1 α , IL-17F, IL-18, IL-22, Lumican, MMP12, MMP3, Serpin E1, SHBG, sIL-6Ra, sRAGE, NT3, α 1-microglobulin and LBP (Bio-Techne, Minneapolis, Minnesota, USA). In this analysis, only sIL6Ra was used. Analyses were performed according to the manufacturers' instructions and measurements were made using the Bio-Plex[®] 200 Luminex system (BIORAD, Hercules, California, USA). Each plate included a randomised mix of samples from each U-BIOPRED subject group. Data from each plate were normalised according to the whole group median for each analyte so that the medians for each plate were equal, and results below the LLQ were replaced with LLQ/2.

hsCRP

Serum CRP levels were measured using a standardised high sensitivity assay at the Centre for Clinical Laboratory Studies, Karolinska University Hospital, Stockholm, Sweden. Samples were analysed using a clinical Cobas c502 (8000) instrument (Roche Diagnostics, Indianapolis, USA), following a particle-enhanced immunoturbidimetric assay. No transformation of raw data was performed other than that results below LLQ were replaced with LLQ/2, which was 0.1 mg/L.

Patient stratification using IL6R levels

Correlation between IL6R sputum mRNA levels and all the other probes in the data was computed using the spearman ranking metric in R. Probes correlated with IL6R with coefficients higher than 0.6 were used as an input to hierarchical clustering. We have then performed a clustering analysis in T-MEV³ to group patients and genes with an average linkage clustering method and using pearson correlation as a distance metric. Pathway analysis was performed with Thomson-Reuters MetaCore[™] software suite (version 6.24)⁴ and representative pathways with FDR<0.1 were reported.

Statistical analysis of clinical data between severe asthma patients with high and low IL6R groups

A t-test was performed for numerical variables in the clinical data comparing the two groups in severe asthma in T-MEV³. Categorical variables, including sex, smoking history, and subjects taking OCS were tested using fisher's exact test in R version 3.2.2⁵.

Statistical analysis of transcriptomics data from sputum

We have identified genes that are differentially expressed between asthma vs. healthy, between severe asthma high IL6R group vs. low IL6R group were identified using a t-test in arrayStudio⁶. The p-values were corrected with Benjamni-Hochberg correction. Cut off of 10% FDR and FC of 1.5 has been used for selecting genes significantly different between the two groups. In Table 1, only p-value column has been reported.

Statistical analysis of proteomics data from blood and sputum

Proteins that are statistically different between low and high IL6R groups were identified using a t-test in arrayStudio⁶. Results of IL6, IL6R, sIL6, CRP were reported in Table 1.

References

1. Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur. Respir. J* 2015; 46:1308-21.
2. Lefaudeux D, De Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. *J Allergy Clin Immunol* 2016.
3. Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, et al. TM4: a free, open-source U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics

system for microarray data management and analysis. Biotechniques 2003; 34:374-8.

4. Reuters T. Metacore. 2016.

5. R: A Language and Environment for Statistical Computing. 2008., 2008.

6. OmicSoft®. OmicSoft® ArraySuite® software., 2016.