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TREM-1 Response Signatures Common to Expression Profiles of Both Asthma Affection and Asthma Control

To the Editor:

It is an essential component of the scientific process to support a hypothesis by demonstrating the convergence of separate lines of evidence generated by independent research groups. In disease genomics, where high-dimensional data have many more features than samples ($p \gg n$), researchers must be especially sensitive to multiple-testing burdens and model overfitting. The case has been made in the *Journal* that gene expression profiling results in a particular need to be approached cautiously until confirmation can be made in other contexts (1, 2). To this end, we read with great interest the report by Bigler and colleagues, from the U-BIOPRED (Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes) study, on the topic of whole-blood gene expression signatures of asthma severity (3). The authors noted that their severe asthma disease signature included three genes related to TREM-1 (triggering receptor expressed on myeloid cell-1) signaling (*CCL23* [C-C motif chemokine ligand 23], *OLIG* [oligodendrocyte transcription factor]-1, and *OLIG2*), which we recently identified as part of a signature of suboptimal asthma symptom control in the Asthma BRIDGE (Asthma Biorepository for Genomic Exploration) cohort (4). Given that the severe asthma disease signature was strongly present in mild and moderate asthma cases, it could also be viewed as a global signature of asthma affection (case) status, regardless of severity. We thus hypothesized that there was, in fact, even more substantive overlap

This work was funded by the NHLBI of the NIH (K01 HL127265 [D.C.C.-C.] and R01 HL118455 and R01 HL086601 [B.A.R.]).

Originally Published in Press as DOI: 10.1164/rccm.201709-1964LE on March 23, 2018

Table 1. Characteristics of the U-BIOPRED (Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes) Study Cohort

	Healthy, Nonsmoking	Moderate Asthma, Nonsmoking	Severe Asthma, Nonsmoking	Severe Asthma, Smoking	P Value
N	87	77	246	88	
Female, n (%)	34 (39.1)	37 (48.1)	161 (65.4)	43 (48.9)	<0.001
Self-reported race, n (%)					0.232
White European	80 (92.0)	72 (93.5)	215 (87.4)	84 (95.5)	
Southeast Asian	0 (0.0)	2 (2.6)	1 (0.4)	0 (0.0)	
Other	0 (0.0)	0 (0.0)	5 (2.0)	0 (0.0)	
Black African	3 (3.4)	3 (3.9)	8 (3.3)	1 (1.1)	
South Asian	1 (1.1)	0 (0.0)	7 (2.8)	2 (2.3)	
Arabic/North African heritage	0 (0.0)	0 (0.0)	5 (2.0)	0 (0.0)	
Multiple races	2 (2.3)	0 (0.0)	4 (1.6)	1 (1.1)	
Central Asian	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	
East Asian	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	
RIN, median (IQR)	9.00 (8.50–9.20)	9.00 (8.80–9.30)	8.75 (8.20–9.10)	8.75 (8.28–9.10)	<0.001
WBC, median (IQR)	5.33 (4.70–6.40)	5.80 (5.10–7.20)	7.79 (6.30–9.40)	7.94 (6.74–9.83)	<0.001
Eosinophils, %, median (IQR)	2.17 (1.50–3.12)	2.90 (1.88–4.55)	2.90 (1.40–6.17)	2.88 (1.58–5.14)	0.012
Lymphocytes, %, median (IQR)	31.20 (26.12–37.91)	30.53 (26.40–33.33)	26.51 (18.70–31.09)	27.55 (21.27–32.98)	<0.001
Monocytes, %, median (IQR)	7.40 (6.12–9.15)	7.27 (5.80–8.95)	6.78 (5.39–8.36)	6.79 (5.08–8.53)	0.026
Neutrophils, %, median (IQR)	57.14 (50.96–63.52)	56.86 (52.20–63.40)	61.11 (54.90–69.03)	60.39 (54.64–68.01)	<0.001

Definition of abbreviations: IQR = interquartile range; RIN = RNA integrity number; WBC = white blood cells (thousands per microliter).

For categorical variables, differences between groups were compared using a chi-squared test with continuity correction. For continuous variables, differences between groups were compared using a Kruskal-Wallis rank sum test.

between our respective gene expression signatures of asthma affection and asthma control, particularly in relation to TREM-1 and LPS signaling, which act together to promote granulocytic inflammation.

To address this overlap hypothesis, we downloaded the U-BIOPRED whole-blood expression data from the Gene Expression Omnibus (accession no. GSE69683) and performed differential gene expression (DGE) analysis in those data, followed by gene set enrichment analysis, using our published TREM-1 and LPS signatures of asthma control. To ensure fidelity of our analysis with the published U-BIOPRED regression models, we obtained additional covariates relevant for testing for DGE from the U-BIOPRED Consortium. In addition, missing metadata values were median-imputed to ensure maximum sample size. A total of 54,715 probes were available for analysis. We generated DGE models using all 498 U-BIOPRED subjects (Table 1), comparing asthmatic cases with control patients, adjusting for the following covariates: sex, severity, smoking status, the first two principal components of gene expression (in place of race [5]), RNA quality, clinical site, total white blood cell counts, and peripheral blood cell differentials (percentage eosinophils, lymphocytes, monocytes, and neutrophils). We then performed fast preranked gene set enrichment analysis (6) on these DGE results to test for enrichment of our reported asthma control signatures ($n = 100,000$ permutations). Specifically, we tested as hypotheses our published leading edges of 24 gene sets (each containing 15–200 gene members) related to exposure-induced TREM-1 and/or LPS signaling in monocytes (derived from the Gene Expression Omnibus dataset GSE9988) (4). We have posted our analysis code on GitHub (<https://www.github.com/dccc-lab/AsthmaGXSigns/>).

We found strong evidence of overlap between the U-BIOPRED gene expression signatures of asthma affection status and our signatures of suboptimal asthma symptom control from Asthma BRIDGE. A total of 1,177 gene probes were differentially expressed between all 411 asthma cases and 87 healthy controls at false discovery rates less than 5%. Among genes with increased expression in asthma

cases, we found significant enrichments for all 24 TREM-1/LPS signatures of asthma control (Figure 1). Together, these gene sets implicated a core of 210 leading-edge genes driving the enrichments, including *CCL23*, *GFOD1* [glucose-fructose oxidoreductase domain containing 1], *OLIG1*, *OLIG2*, and *HSD3B7* [hydroxy- Δ -5-steroid dehydrogenase, 3 β - and steroid Δ -isomerase 7]: five genes specifically highlighted in our previous work on asthma control (4). The 24 significant gene set enrichments were largely robust to multiple sensitivity analyses, including one that excluded 88 severe smokers, a second that limited the contrast to the 77 moderate cases versus controls, and a third that limited the comparison to the 246 nonsmoking severe cases versus controls (only here did one set not reach false discovery rate <5%). Notably, significant enrichment was not observed in an asthma-only comparison of the nonsmoking severe cases with the mild/moderate cases, suggesting that the TREM-1/LPS pathway genes associate with asthma affection status and asthma control, regardless of asthma severity.

These results advance the relevance of the TREM-1/LPS response pathway to asthma pathobiology in two important ways. First, the availability of control patients without asthma in these analyses extends our prior work in Asthma BRIDGE, which was limited to only patients with asthma (4). As such, we were able to demonstrate that in addition to the importance of the TREM-1/LPS response pathway in asthma to disease control, the pathway may also be of relevance to disease pathogenesis. Second, and perhaps more important, the analysis presented here emphasizes a broad degree of comparable gene expression between affection and control signatures, beyond the three-gene overlap highlighted by Bigler and colleagues (3). By demonstrating the shared enrichment for a specific pathway in two disparate data sets, we have more confidence that we have identified a robust core of genes, related through a specific biological process, relevant to the manifestation of asthma phenotypes. These core genes merit further functional characterization and investigation as therapeutic targets.

Figure 1. TREM-1 (triggering receptor expressed on myeloid cell-1)/LPS response gene sets associated with asthma control enriched for expression in asthma cases. Each bar graph shows the distribution of individual gene set members among all differential gene expression results comparing U-BIOPRED (Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes) asthma cases to controls. Results are arranged from left to right by decreasing expression in cases versus controls. FDR = false discovery rate; GSE9988 = Gene Expression Omnibus dataset entitled “Innate immune responses to TREM-1 activation”; NES = normalized enrichment score.

Our work here provides a small window into the enormous potential and value of publicly accessible biological data. Genomic analyses of openly shared data will continue to inform us about the biological relationships of related respiratory disease phenotypes (7), and it is only through unfettered access to large, well-characterized datasets that the scientific community will be able to quickly make advances in pulmonary genomics and precision medicine. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Acknowledgment: The authors thank the U-BIOPRED Consortium for making their gene expression data publicly available for further analysis and for providing access to the additional metadata.

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Lung Ultrasound and Microbubbles Enhance Aminoglycoside Efficacy and Delivery to the Lung in *Escherichia coli*-induced Pneumonia and Acute Respiratory Distress Syndrome

To the Editor:

Acute respiratory distress syndrome (ARDS) is a devastating disorder characterized by lung microvascular leakage leading to pulmonary edema and hypoxemia (1). Its hallmark is heterogeneous involvement of the lung parenchyma, with thoracic computed tomographic scans revealing densely consolidated areas interspersed with relatively normal-appearing lung. Such heterogeneity greatly complicates management. Mechanical ventilation, although lifesaving, preferentially inflates the normal regions of the lung, causing overdistention and lung damage (1). Pharmaceutical agents such as antibiotics administered by

inhalation distribute to the healthy rather than the injured regions of the lung, potentially causing off-target effects. By contrast, drugs administered systemically cannot target the injured lungs and are delivered to organs throughout the body, thereby predisposing to adverse effects (2). The inability to deliver therapy solely to the injured areas of the ARDS lung remains an intractable problem in critical care.

We hypothesized that thoracic ultrasound and microbubbles could be used to specifically enhance drug delivery to and uptake by the injured lung. Microbubbles (1–3 μm) are made up of a gas-filled core and an outer lipid shell and are used routinely as a contrast agent for echocardiographic studies, including in critically ill patients (3). When exposed to an ultrasound pulse, microbubbles undergo cavitation that induces shear stress on biological membranes within their vicinity, leading to the formation of transient pores and enhanced endocytosis (4) at the plasma membranes of cells. This induces enhanced uptake of local therapeutics such as drugs or genes (5); importantly, the process is effective at increasing uptake whether the drug is encapsulated by the microbubble, bound to its surface, or even only in its vicinity. This phenomenon has been harnessed to deliver genes to ischemic limbs (5). Ultrasound of the lung has traditionally been considered of limited utility (6) because air causes reflection and scattering of ultrasound energy. We hypothesized that this very limitation can be harnessed in ARDS because ultrasound waves will preferentially penetrate damaged areas of lung (because they are filled with fluid or are atelectatic), leaving normal (air-filled) areas of lung mostly unaffected (Figure 1A). In addition, by targeting the ultrasound transducer to the chest, the ultrasound energy is focused on the thorax, limiting the effect on other organs. Furthermore, circulating microbubbles may accumulate in injured regions owing to increased endothelial permeability.

To test this hypothesis, we focused our initial efforts on aminoglycoside antibiotics using a murine model of severe gram-negative bacterial pneumonia, one of the commonest causes of ARDS. Antibiotic resistance is growing worldwide (7); many strains of enteric gram-negative bacilli are now resistant to cephalosporins and β -lactam/ β -lactamase inhibitor combinations. Although almost 100% of gram-negative isolates remain sensitive to aminoglycosides, this class of antibiotics has traditionally been avoided for treating pneumonia because of poor lung penetration and the association of systemic therapeutic aminoglycoside concentrations with significant nephrotoxicity and ototoxicity (8). Thus, developing an easy and safe method to increase the therapeutic index of aminoglycosides specifically in the lungs would be of great practical value.

C57BL/6 mice (male; aged 9–12 wk) were infected intratracheally with *Escherichia coli* (American Type Culture Collection 25922) and developed significant hypoxemia and lung edema within 3 hours; histological examination confirmed extensive neutrophilic lung injury that was bilateral (Figure 1B). All work was performed with the approval of the local animal care committee (ACC 742).

To confirm the safety of microbubbles and ultrasound-mediated cavitation in this setting, we measured oxygen saturation by pulse oximetry before, during, and up to 48 hours after intravenous injection of 1×10^9 DEFINITY microbubbles (Lantheus Medical Imaging; dose based on previous work [5]) and thoracic ultrasound administration; no antibiotics were

Supported by the Canadian Institutes of Health Research (MOP 130564 [W.L.L.]), the Canada Foundation for Innovation (CFI 34769 [W.L.L.]), a Natural Sciences and Engineering Research Council (NSERC) of Canada Engage award (503493-16 [W.L.L.]), and an Ontario Centres of Excellence Voucher for Innovation and Productivity I award (OCE-VIP1 28176 [W.L.L.]). This work was also partially supported through the Institute for Biomedical Engineering Science and Technology (iBEST), a partnership between Ryerson University and St. Michael's Hospital. W.L.L. holds the Canada Research Chair in Mechanisms of Endothelial Permeability.

Author Contributions: Conception and design: W.L.L., R.K., H.L.-P., and W.M.K.; acquisition, analysis, or interpretation of data: M.G.S., V.M., H.R., N.M.G., J.E.B., L.B., and W.L.L.; drafting of the manuscript for important intellectual content: W.L.L., W.M.K., and L.B.

Originally Published in Press as DOI: 10.1164/rccm.201711-2259LE on April 11, 2018