Transcriptome Analysis of Airway Brushes in Lung Transplant Recipients with and without Chronic Lung Allograft Dysfunction

C Iasella, M Snyder, I Popescu, J Wei, A Hoji, Y Zhang, W Xu, V Iouchmanov, M Brown, E Lendermon, B Johnson, S Kilaru, M Morrell, J Pilewski, J Greenland, K Chen, J McDyer

University of Pittsburgh

UPMC
LIFE CHANGING MEDICINE

Disclosures

- I will not discuss off label use and/or investigational use of drugs/devices
- All authors have no relevant financial relationships related to this presentation

Background

- Chronic lung allograft dysfunction (CLAD) is the major complication limiting long-term survival in lung transplant recipients (LTRs)
- CLAD is diagnosed using spirometry and the exclusion of other pathologies and generally responds poorly to augmentation of conventional immunosuppressive therapy
- The immunopathogenesis of CLAD and its clinical endotypes remain poorly understood
- There is a major need in the lung transplant field to identify novel early molecular pathways to diagnose early CLAD

Purpose

- To address the pathogenesis of CLAD at the molecular level, we performed RNA-seq analysis of airway brush samples in LTRs with and without CLAD

- We hypothesized the airway transcriptome would reflect key immunologic changes in disease

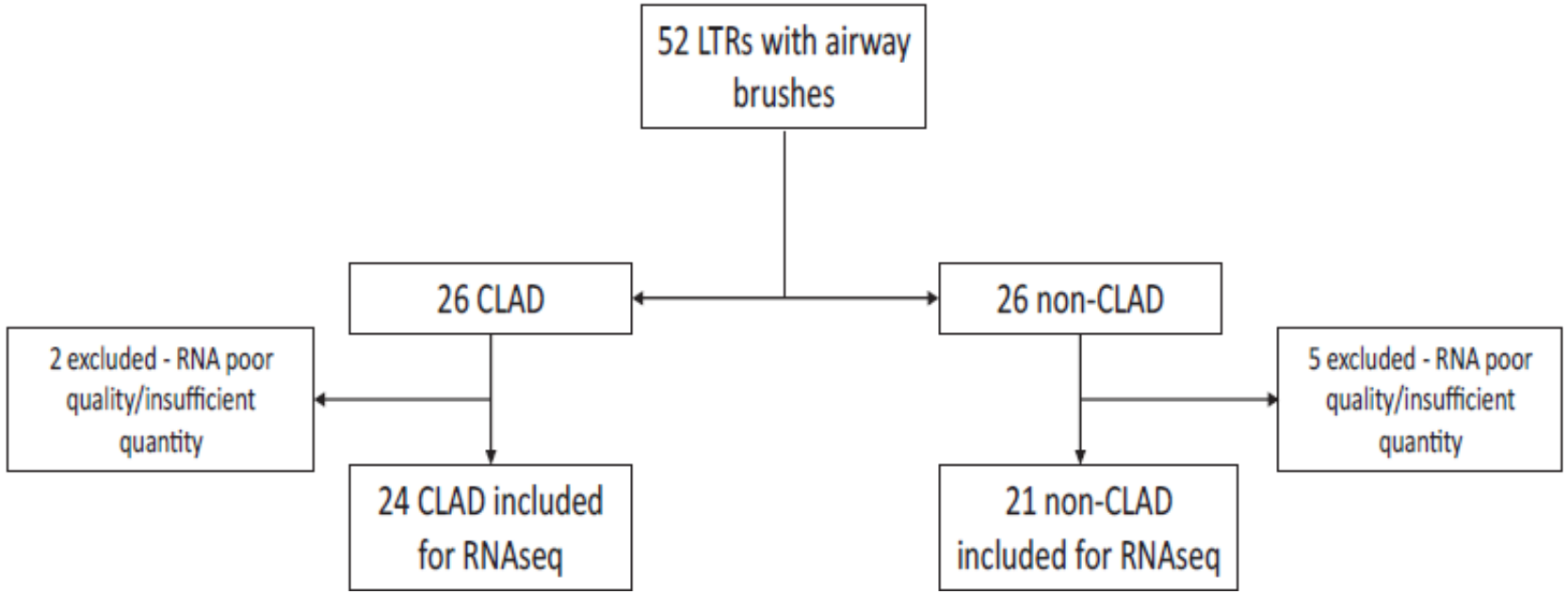
Study Design

- Single center, cross-sectional study
- Patients were lung transplant recipients identified from an institutional registry and biorepository
- Transbronchial brush samples were obtained during clinical bronchoscopies

Methods

- Total RNA was isolated and stranded total RNA-seq libraries were prepared following ribosomal RNA depletion with Illumina reagents
- Libraries were sequenced with an Illumina Nextseq500 sequencer with a depth of paired-end 40 million read pairs per sample
- Reads were aligned to hg38 human genome
- Transcript counts and differential expression analysis were carried out using the CLC Genomics Workbench.

Results



12 brushes from control patients were used to evaluate the cellular populations in brush samples and found that epithelial cells were the predominant population (59%), followed by monocyte/ macrophages (30%), with lymphocytes and NK cells comprising the minority balance of cells

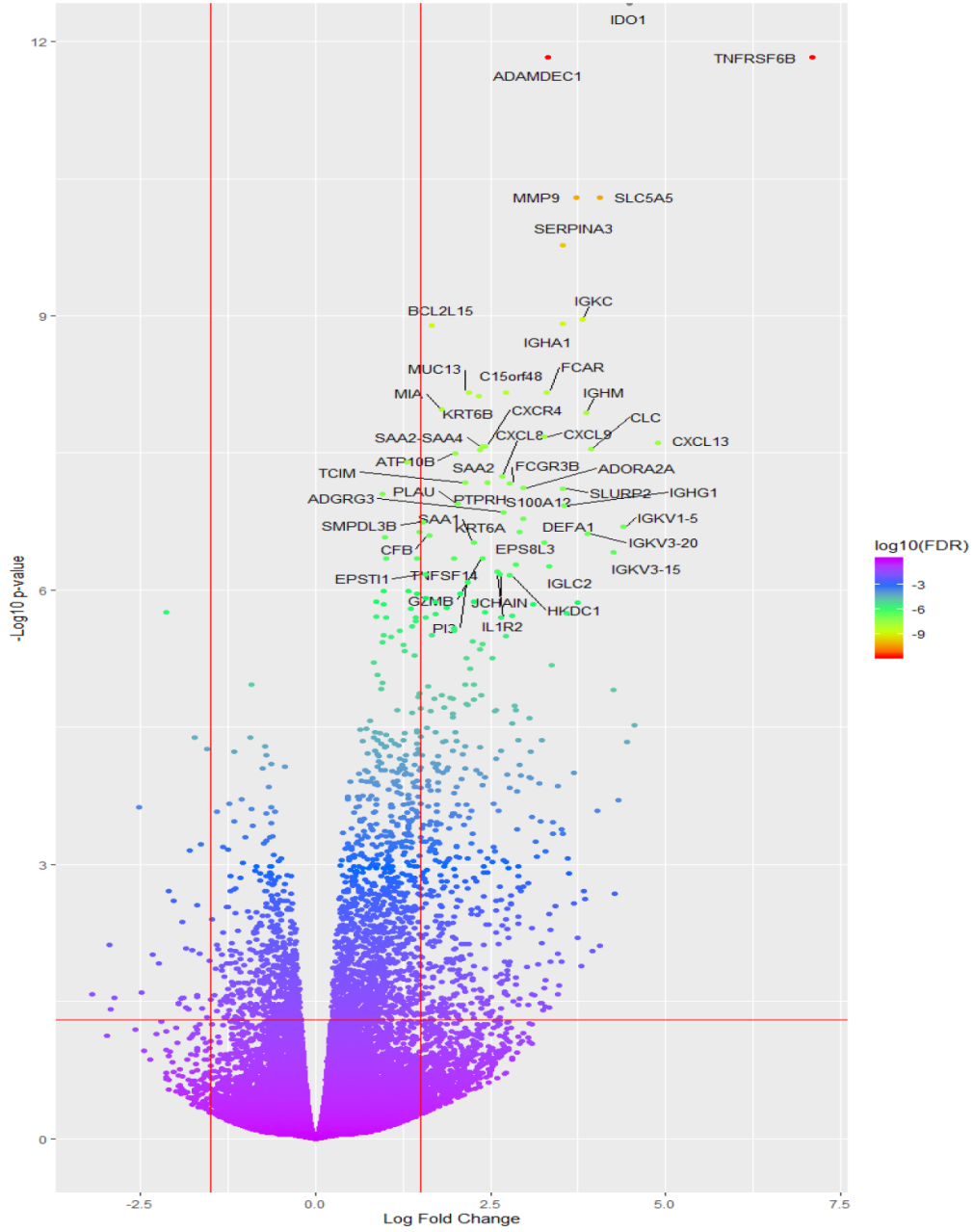
Cohort Characteristics

	CLAD (n=24)	Control (n=21)	p-value
Age at transplant (median, IQR)	52.5 (34.75-62.75)	54 (37.0-63.5)	0.59
Female (n, %)	10 (41.7%)	10 (47.6%)	0.16
Transplant Diagnosis			0.91
Interstitial Lung Disease (n, %)	9 (37.5%)	7 (33.3%)	
Cystic Fibrosis (n, %)	7 (29.2%)	7 (33.3%)	
Chronic Obstructive Pulmonary Disease (n, %)	7 (29.2%)	6 (28.6%)	
Re-transplant (n, %)	1 (4.2%)	1 (4.8%)	
Congenital (n, %)	1 (4.2%)	0	
Acute Rejection (n, %)	9 (37.5%)	3 (14.3%)	0.11
Acute Infection (n, %)	13 (52.4%)	0	<0.01
Bacterial	10	-	
Viral	1	-	
Fungal	2		
Days to CLAD (median, IQR)	1645 (1133-1981)	-	
CLAD Stage (n, %)			
CLAD 1	15 (62.5%)	-	
CLAD 2	3 (12.5%)	-	
CLAD 3	6 (25.0%)	-	
CLAD 4	0		

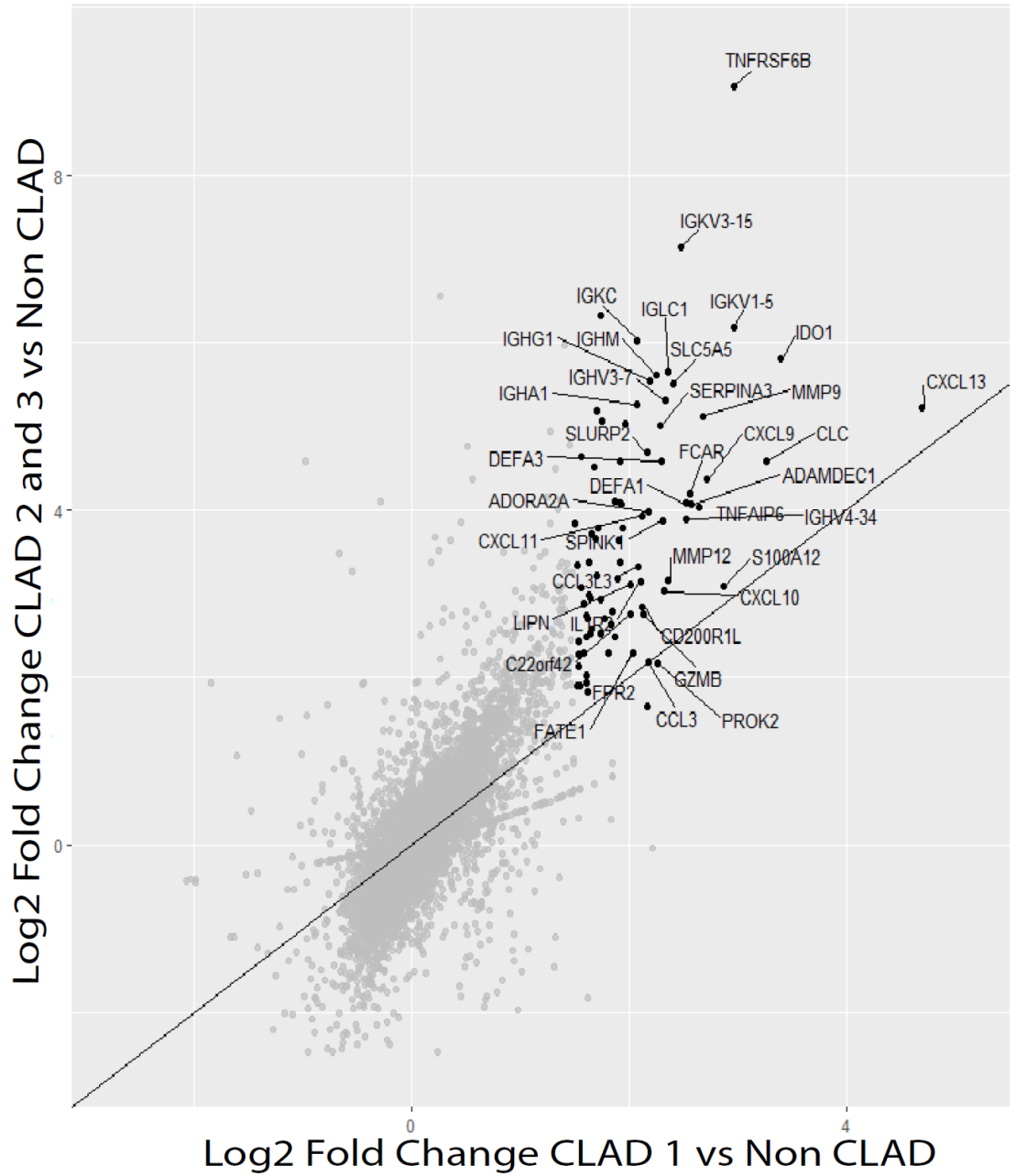
Top 25 Upregulated Genes

CLAD vs Non-CLAD		
Gene	**Fold Change**	**FDR p-value**
IDO1	22.32	<1.00E-13
ADAMDEC1	9.99	1.49E-12
TNFRSF6B	137.19	1.49E-12
SLC5A5	16.68	5.10E-11
MMP9	13.18	5.10E-11
SERPINA3	11.63	1.69E-10
IGKC	14.03	1.10-09
IGHA1	11.55	1.21E-09
BCL2L15	3.18	1.28E-09
MUC13	4.56	6.84E-09
C15orf48	6.59	6.84E-09
FCAR	9.92	6.84E-09
KRT6B	5.03	7.54E-09
MIA	3.51	1.05E-08
IGHM	14.52	1.16E-08
CXCL9	9.65	2.11E-08
CXCL13	29.65	2.47E-08
CXCR4	5.35	2.66E-08
SAA2-SAA4	5.21	2.66E-08
CLC	15.35	2.88E-08
SAA2	5.10	2.94E-08
ATP10B	4.00	3.18E-08
CXCL8	6.41	5.68E-08
TCIM	4.41	6.73E-08

A


IDO1
TNFRSF6B
ADAMDEC1
MMP9
SLC5A5
SERPINA3
IGKC
BCL2L15
IGHA1
MUC13
C15orf48
FCAR
MIA
IGHM
KRT6B
CXCR4
CLC
SAA2-SAA4
CXCL8
CXCL9
CXCL13
ATP10B
SAA2
FCGR3B
ADORA2A
TCIM
PLAU
PTPRH
S100A12
SLURP2
IGHG1
ADGRG3
SAA1
KRT6A
DEFA1
IGKV1-5
SMPDL3B
CFB
EPS8L3
IGKV3-20
IGKV3-15
EPSTI1
TNFSF14
IGLC2
GZMB
JCHAIN
HKDC1
PI3
IL1R2
log10(FDR)
-3
-6
-9
-Log10 p-value
Log Fold Change
12
9
6
3
0
-2.5
0.0
2.5
5.0
7.5

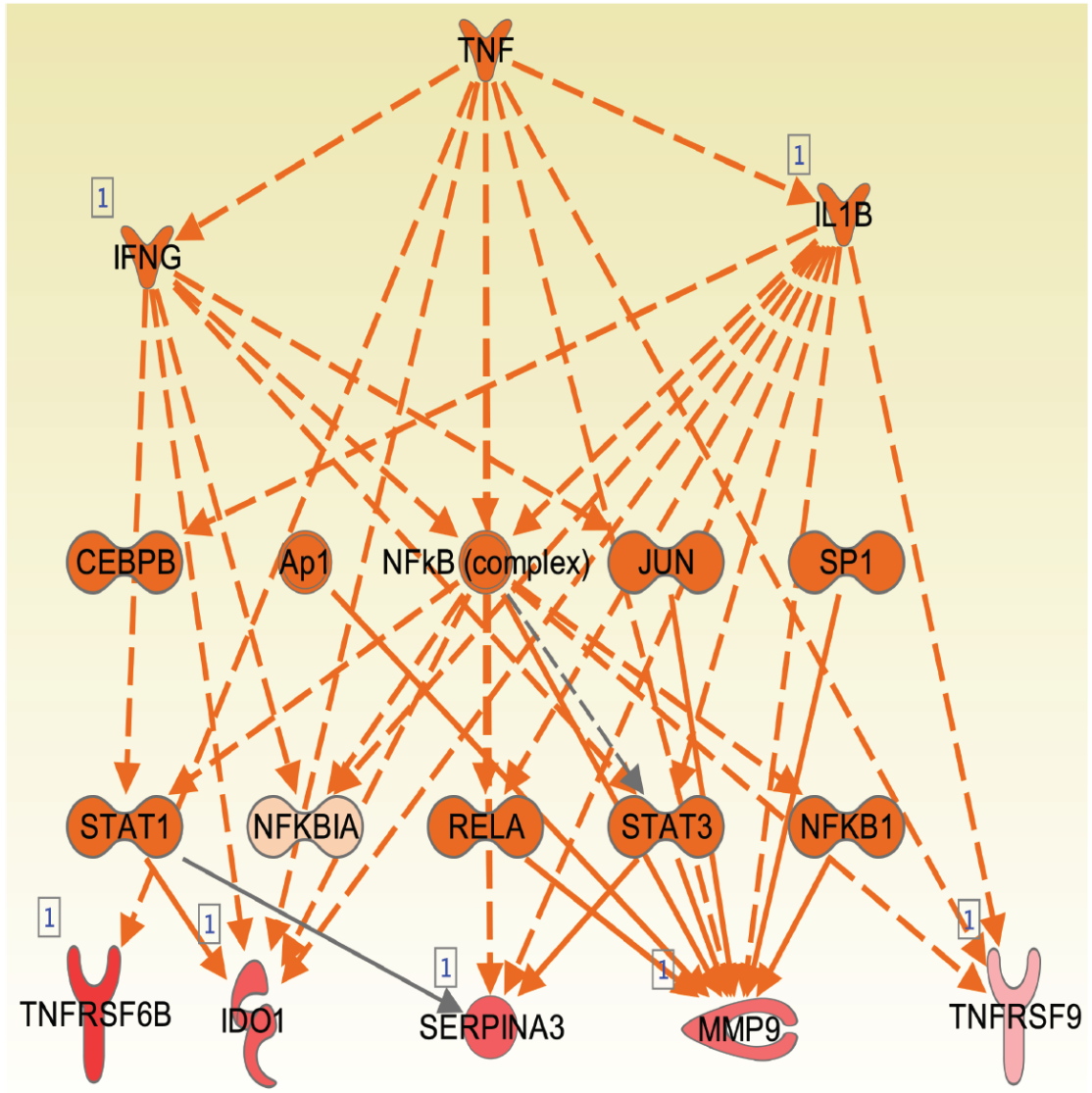
B


TNFRSF6B
IGKV3-15
IGKC
IGLC1
IGKV1-5
IGHG1
IGHM
SLC5A5
IDO1
IGHV3-7
IGHA1
SERPINA3
MMP9
CXCL13
SLURP2
CXCL9
CLC
DEFA3
FCAR
ADAMDEC1
DEFA1
ADORA2A
TNFAIP6
IGHV4-34
CXCL11
SPINK1
MMP12
S100A12
CCL3L3
CXCL10
LIPN
IL1R2
CD200R1L
C22orf42
GZMB
FPR2
CCL3
PROK2
FATE1
Log2 Fold Change CLAD 2 and 3 vs Non CLAD
Log2 Fold Change CLAD 1 vs Non CLAD
8
4
0
0
4

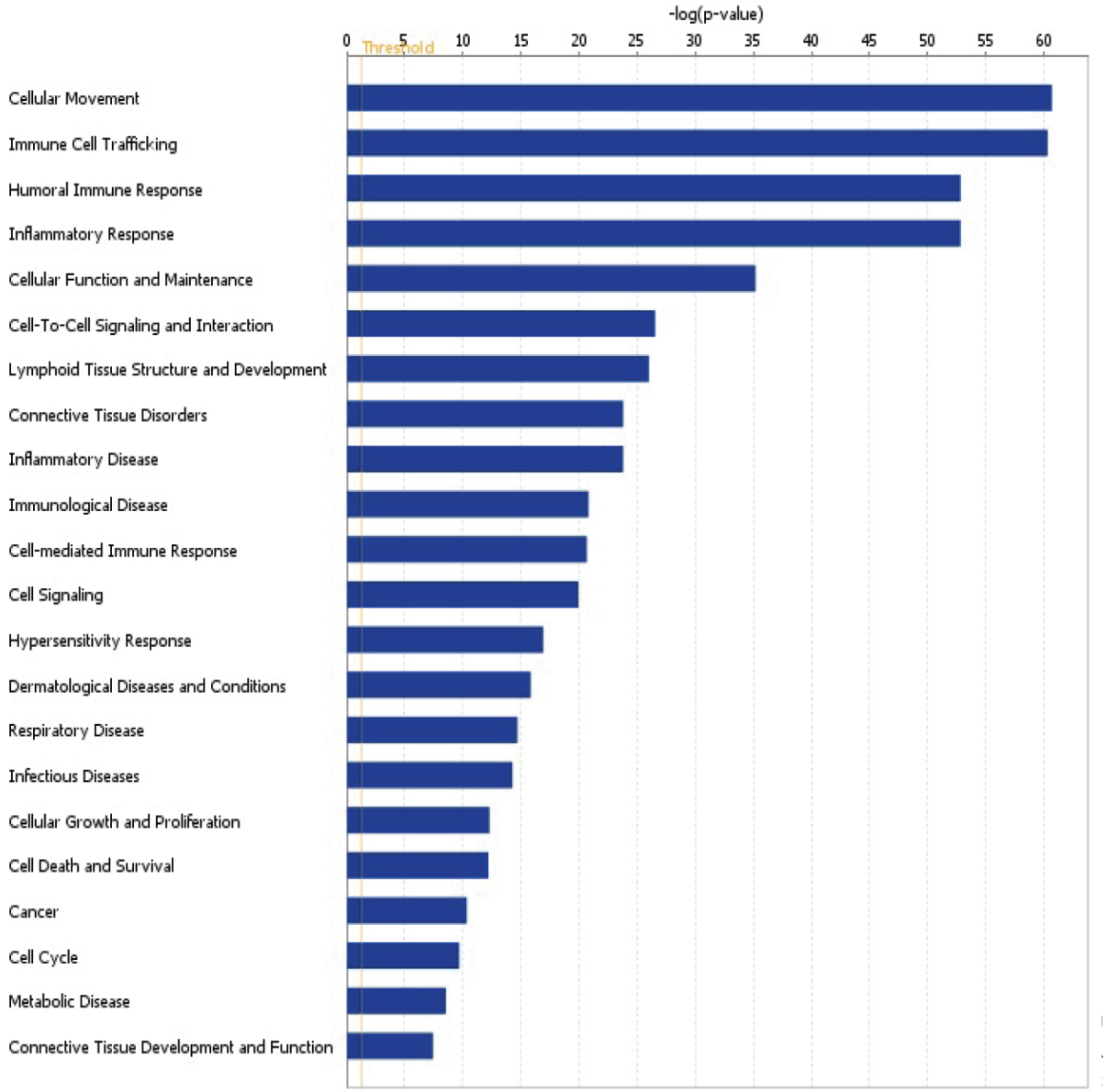
Upstream Regulators

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
TNF-α	Activated	7.017	2E-21
IL1-β	Activated	6.412	7E-30
IFN-γ	Activated	6.013	8E-20
IL-6	Activated	5.941	3E-23
NFκβ (complex)	Activated	5.921	4E-20
IL1-α	Activated	5.308	5E-22
MYD88	Activated	4.957	8E-15
CSF2	Activated	4.793	2E-17
IL-2	Activated	4.631	8E-16
TLR3	Activated	4.565	2E-12

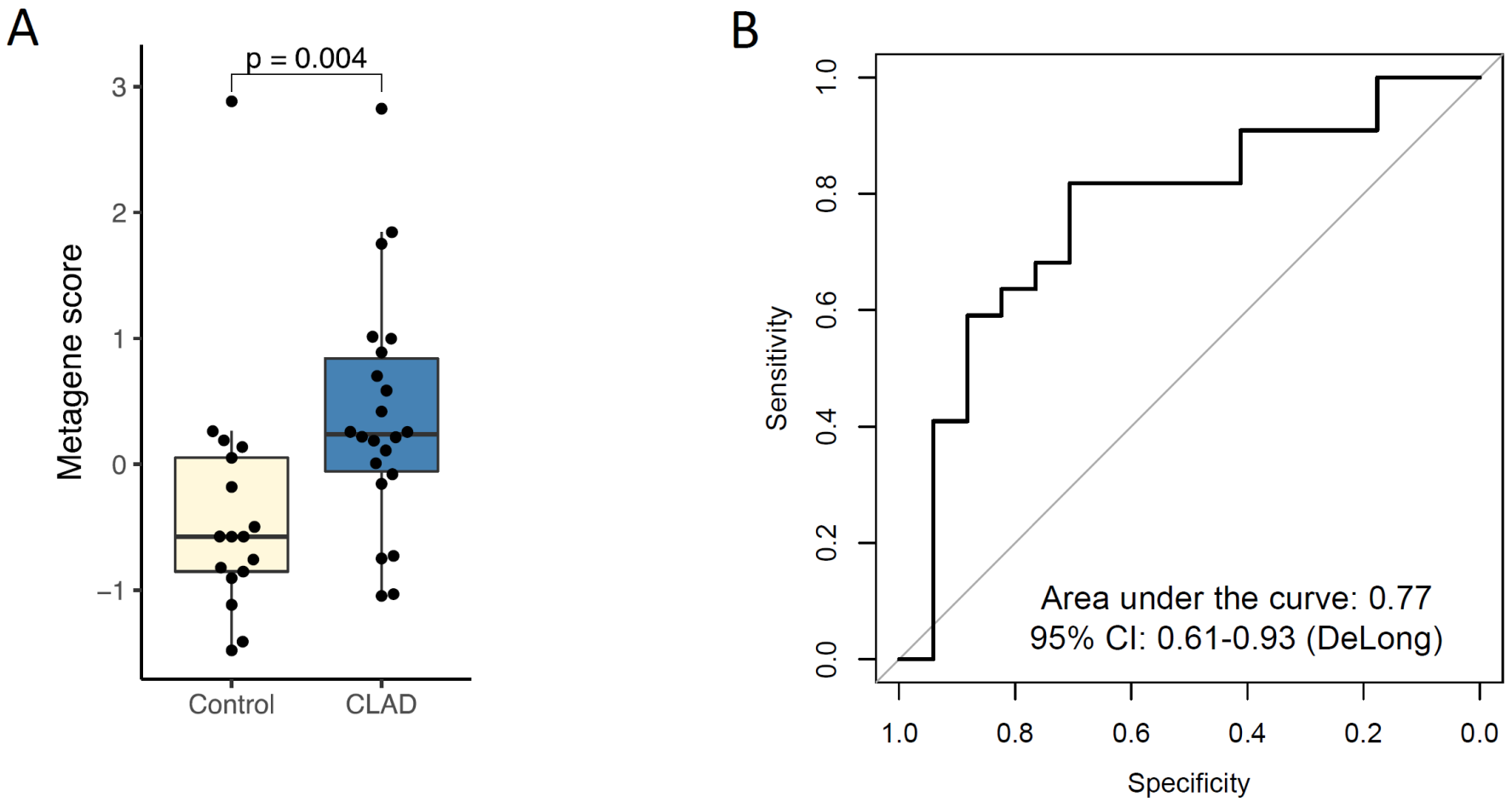
A


TNF
IFNG
IL1B
CEBPB
Ap1
NFkB (complex)
JUN
SP1
STAT1
NFKBIA
RELA
STAT3
NFKB1
TNFRSF6B
IDO1
SERPINA3
MMP9
TNFRSF9
1

B


-log(p-value)
0
5
10
15
20
25
30
35
40
45
50
55
60
Threshold
Cellular Movement
Immune Cell Trafficking
Humoral Immune Response
Inflammatory Response
Cellular Function and Maintenance
Cell-To-Cell Signaling and Interaction
Lymphoid Tissue Structure and Development
Connective Tissue Disorders
Inflammatory Disease
Immunological Disease
Cell-mediated Immune Response
Cell Signaling
Hypersensitivity Response
Dermatological Diseases and Conditions
Respiratory Disease
Infectious Diseases
Cellular Growth and Proliferation
Cell Death and Survival
Cancer
Cell Cycle
Metabolic Disease
Connective Tissue Development and Function

Validation Cohort



The top 25 genes from the study cohort were used to classify CLAD in the external validation cohort. As a group, these genes were significantly upregulated in the CLAD patients in the validation cohort.

University of Pittsburgh

UPMC
LIFE CHANGING MEDICINE

Conclusions

- The airway transcriptome demonstrated a predominant Type-1 immune activation signature in a cross-sectional study of CLAD versus stable LTRs
- Pathway analysis of the airway transcriptome predicts upregulation of TNF-α, IFN-γ, and IL-1β as the predominant immune mediators
- Further analyses may provide the rationale for testing select immune targets in CLAD as biomarkers and further defining distinct immune endotypes within CLAD