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Genomic and Genetic Approaches to Deciphering Acute Respiratory Distress Syndrome Risk and Mortality

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Abstract

Significance: Acute respiratory distress syndrome (ARDS) is a severe, highly heterogeneous critical illness with staggering mortality that is influenced by environmental factors, such as mechanical ventilation, and genetic factors. Significant unmet needs in ARDS are addressing the paucity of validated predictive biomarkers for ARDS risk and susceptibility that hamper the conduct of successful clinical trials in ARDS and the complete absence of novel disease-modifying therapeutic strategies.

Recent Advances: The current ARDS definition relies on clinical characteristics that fail to capture the diversity of disease pathology, severity, and mortality risk. We undertook a comprehensive survey of the available ARDS literature to identify genes and genetic variants (candidate gene and limited genome-wide association study approaches) implicated in susceptibility to developing ARDS in hopes of uncovering novel biomarkers for ARDS risk and mortality and potentially novel therapeutic targets in ARDS. We further attempted to address the well-known health disparities that exist in susceptibility to and mortality from ARDS.

Critical Issues: Bioinformatic analyses identified 201 ARDS candidate genes with pathway analysis indicating a strong predominance in key evolutionarily conserved inflammatory pathways, including reactive oxygen species, innate immunity-related inflammation, and endothelial vascular signaling pathways.

Future Directions: Future studies employing a system biology approach that combines clinical characteristics, genomics, transcriptomics, and proteomics may allow for a better definition of biologically relevant pathways and genotype–phenotype connections and result in improved strategies for the sub-phenotyping of diverse ARDS patients *via* molecular signatures. These efforts should facilitate the potential for successful clinical trials in ARDS and yield a better fundamental understanding of ARDS pathobiology. *Antioxid. Redox Signal.* 31, 1027–1052.

Keywords: acute respiratory distress syndrome (ARDS), genome-wide association studies (GWAS), ARDS mortality, reactive oxygen species (ROS), inflammation, pathway analysis, candidate gene studies

Introduction

CRITICAL ILLNESSES, INCLUDING infection, sepsis, trauma, pancreatitis, hemorrhage, and acute respiratory failure/acute respiratory distress syndrome (ARDS) (Fig. 1), account for 20% of health care costs in the United States ($90 billion annually) (152, 206). Estimates of the incidence of ARDS vary between ~200,000 and 400,000 individuals annually in the United States with mortality rates of ~30%–40% (23, 40, 49, 51, 64, 129, 176). The pathologic hallmarks of ARDS are marked increases in high permeability pulmonary edema related to acute endothelial cell activation/dysfunction resulting in paracellular gap formation (57, 58, 62), alveolar flooding, decreased lung compliance, severe hypoxemia, and a requirement for mechanical ventilation.

From a pathobiological standpoint, two categories of lung injury are recognized in ARDS. Direct pulmonary injury, which is to the local lung epithelium such as in pneumonia,

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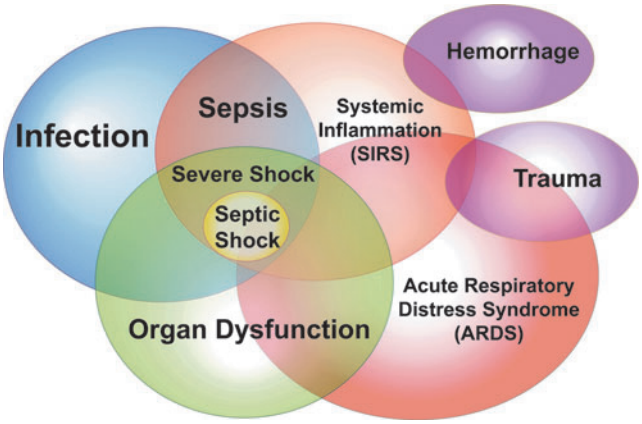

FIG. 1. Schema of the overlapping syndromes and complications in the critically ill. Depicted are the major causes of critical care illnesses and associated complications that contribute to the staggering morbidity, mortality, and health care costs in the critically ill.

aspiration, mechanical ventilation, inhalation injury, and lung contusion. Indirect pulmonary injury occurs secondary to vascular endothelial damage (156), with sepsis being the most common cause of indirect lung injury and the highest contributor to ARDS mortality (Fig. 2) (49). However, these broad categorizations and classifications of ARDS fail to capture the nuances of the disease and the substantial heterogeneity of phenotypes within ARDS has made interrogation of basic mechanisms and therapeutic development extremely challenging. This has contributed to the abysmal track record of Phase II/III trials of novel therapies in ARDS (70, 98, 152). Clinical factors alone have failed to predict which patients will develop ARDS or develop severe ARDS (122).

Although promising attempts exist to sub-phenotype ARDS cohorts *via* blood-derived biomarkers that are predictive of mortality in hope of identifying at-risk individuals, successful validation has been elusive (189–191). Scoring systems such as the acute physiology and chronic health evaluation II score (101, 152) or the injury severity score (130) in critically ill patients predict patient outcomes but can only be applied to general intensive care unit populations and do not provide consistent and accurate estimates of the risk of death in specific intensive care unit (ICU) patient populations. For example, mean lung injury scores were not significantly different between ARDS survivors and non-survivors (42, 104) and attempts to characterize predictors of death in ARDS by developing a prognostic index (189) remain controversial and without replication or validation.

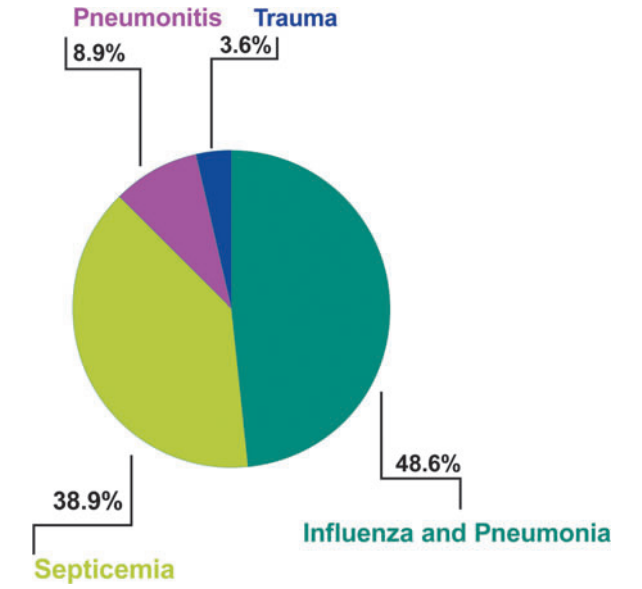

FIG. 2. ARDS causes. An adaptation of the summary of Cochi *et al.* data from 15 years of ARDS comorbidity data (1999–2013) that identified four major comorbidities that occur with ARDS (49). ARDS, acute respiratory distress syndrome.

Thus, there is a compelling unmet need to identify ARDS sub-phenotypes that risk-stratify patients for both accurate prognostication and clinical trial purposes. A risk score is a standardized metric for the likelihood that an individual will experience a particular outcome—in this instance, higher risk for ARDS mortality (32, 189). Risk stratification is the aggregation of multiple individual risk scores to create a broader, more complex profile of risk.

The ability to leverage newly described systems biology and ''omic'' approaches and techniques (genetic and biomarker panels) hold promise for improving the capacity to characterize risk and prognosis for ARDS and in other ICU patients with critical illness and respiratory failure. ARDS genetic and genomic studies potentially provide the basis for identifying candidate genes, biomarker discovery, risk stratification, and novel ARDS therapeutic targets (83, 122). Although ARDS is not a known inheritable condition, the pattern of injury response-recovery has significant heritability (121, 122, 197). Unlike rare and high penetrance monogenetic diseases, ARDS risk and severity are influenced by multiple genes to a varying effect.

The potential to utilize genomic approaches to identify ARDS high inflammatory sub-phenotypes at higher risk for death is a currently untapped area of therapeutic stratification in severe lung injury (32, 65, 122, 149, 175). The diversity of potential genetic biomarkers in ARDS ranges from markers of epithelial injury (the receptor of advanced glycation end products [RAGE]) (62, 97), endothelial activation/injury (angiopoetin [ANGPT-1], intercellular adhesion molecule 1 [ICAM-1], vascular endothelial growth factor [VEGF]) (33, 60, 62, 133, 159), pro-inflammatory (interleukin [IL]-1B, IL-18, IL-6, IL-8) (55, 68, 79, 118, 120, 140, 166, 183), anti-inflammatory molecules (IL-10) (140), coagulation and fibrinolysis proteins (pediocin PA-1) (28, 37, 56, 144), and macrophage markers (high mobility group box 1 [*HMGB1*], macrophage migration inhibitory factor [*MIF*]) (27, 50, 71, 74, 131).

Significance

We (Oita *et al.*, manuscript in preparation, 3, 23–26, 35, 46, 59, 76, 82, 90, 126, 132, 141, 157, 169, 187, 199, 202, 205) and others (2, 13, 19, 21, 22, 45, 47, 63, 69, 92, 122–124, 149, 153, 163, 173, 181, 194) have contributed to the notion that ARDS represents the ultimate in genetic stress, with a complex assortment of genes that contribute a limited overall effect size per gene (149). Individually, many of these loci have limited value for risk prediction, but their aggregated impact on lung injury phenotypes (effect size) in ARDS pathology is much greater (122).

Unlike other complex genetic diseases, ARDS has not benefited from family pedigree studies (149); however, using both candidate gene and genome-wide association study (GWAS) approaches, we have identified several novel genetic targets and biomarkers of ARDS risk and severity, including nicotinamide phosphoribosyl transferase (*NAMPT*) (108, 168, 199), toll-like receptor 4 (*TLR4*) (169, 200), encoding myosin light chain kinase (*MYLK*) (46, 47, 72, 73, 119, 187), iodothyronine deiodinase *2* (*DIO2*) (187, 194), growth arrest and DNA damage-inducible gene (*GADD45a*) (124, 126), *MIF* (71), and sphingosine 1-phosphate receptors 1 and 3 (*S1P1*, *S1P3*) (132, 169).

In this article, we have chosen to integrate studies utilizing peripheral blood mononuclear cells (PBMCs) for identification of genetic signature in ARDS, meta-analysis of ARDS risk, and mortality biomarker studies (175) with GWAS. We speculate that this strategy may expand the understanding of ARDS pathobiology and potentially identify genes associated with ARDS mortality that may serve as diagnostic makers and therapeutic targets.

We evaluated PubMed literature relevant to ARDS to identify a total of 201 dysregulated genes potentially associated with either ARDS risk or severity followed by pathway analysis. Pathway analysis included genes from both candidate and agnostic GWAS studies, genes from mRNA microarray and sequencing studies, and proteomic evaluations that identified putative candidate genes but without associated single nucleotide polymorphisms (SNPs) related to ARDS risk or ARDS mortality. Blood biomarkers without genomic/genetic evidence were not included.

We further summarize the current state of ARDS genetics in terms of susceptibility risk SNPs and those that confer increased mortality selected on the basis of adjusted significance in their respective study. We have also chosen to evaluate ARDS studies that use mortality as an end-point as this captures the most severe outcome for ARDS patients. Our bioinformatically derived results are consistent with the concept that evolutionarily conserved inflammatory networks comprising reactive oxygen species (ROS), innate immunity-related inflammation, and endothelial vascular signaling pathways are potent contributors to multiple organ dysfunction-related ARDS mortality and pathobiology (82, 83).

Recent Advances

A brief timeline of ARDS clinical and genetic literature

ARDS was first described in the classic Ashbaugh *et al.* report in 1967 (10). Clinical therapeutics in ARDS have been studied exclusively in ARDS for several decades (107). Figure 3 depicts a brief timeline of the initial important clinical studies for common therapeutics in ARDS. The two earliest clinical methods to manage ARDS were management of lung collapse utilizing positive end-expiratory pressure and the prone position to aid oxygenation (30a, 171). Ventilator-induced lung injury (VILI) or barotrauma was later described, and the further lung injury and alveolar rupture induced by the mechanical ventilator used to treat ARDS patients provides an additional challenge in the clinic (57). In 2000, the landmark ARDSNet clinical trial using lower tidal volumes to reduce VILI and ARDS mortality represented another significant clinical therapeutic advance (8) (Fig. 3), the sole ARDS clinical trial to succeed.

In contrast, the first candidate gene study in ARDS involving angiotensin-converting enzyme (*ACE*) polymorphisms corresponding to higher ACE plasma levels in ARDS was reported in 1992. Candidate gene studies in ARDS, rare until the sequencing of the human genome (177), have subsequently significantly populated the ARDS literature (Oita *et al.*, manuscript in preparation, 2, 3, 9, 11, 33, 36, 52,

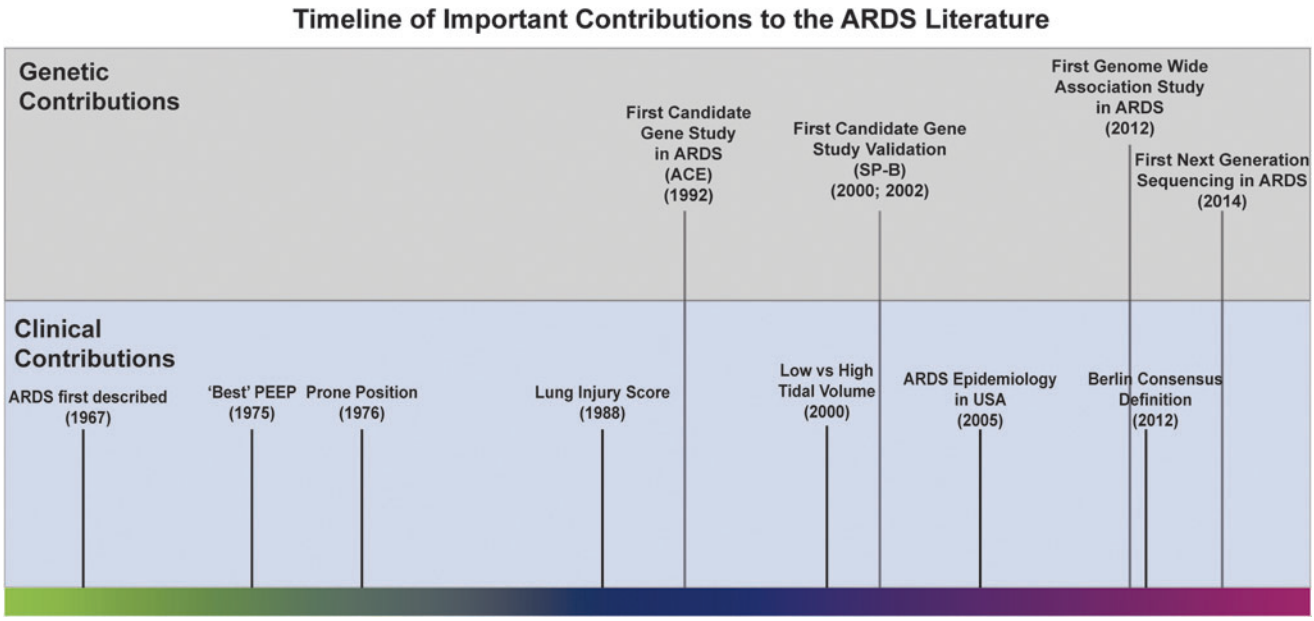

FIG. 3. A timeline of ARDS clinical and genetic contributions. A selection of clinical contributions for ARDS (*bottom*) and genetic contributions to the ARDS literature (*top*). The relative recent history of genetic contributions to the ARDS literature should be noted compared with a much long history of clinical contributions to treatment. Clinical and genetic timelines adapted from Laffey *et al.* and Reilly *et al.* (107, 149).

61, 79, 89, 91, 98, 105, 106, 111, 112, 116, 120, 123, 127, 128, 131, 134, 140, 147, 150, 160, 168, 169, 173, 174, 183, 186, 187, 191–194, 198, 201, 203). The chronology of the discovery of the major candidate genes in ARDS as well as the transition into ARDS GWAS studies has been previously elegantly detailed (149); however, in this review, we have attempted to capture more recent genetic developments and reporting in ARDS (53, 107).

We integrated a variety of genetic studies into the ARDS literature, and excluding the initial ACE polymorphism study (177), all candidate gene studies were published after 2000 (Fig. 3). GWAS studies enter the ARDS literature starting in 2012, but compared with GWAS studies in other disorders and clinical arenas, ARDS GWAS studies generally include smaller cohorts (122). The literature of ARDS genetics is relatively small and recent compared with other academic fields, and the genes presented are a comprehensive list of all mapped genes in ARDS that were significant (irrespective of the individual study) for either (i) a specific polymorphism associated with ARDS or (ii) an overall gene expression level significant for ARDS risk.

Later, the importance of gene expression studies and PBMC mortality risk genes are discussed as these genes potentially serve as novel therapeutic targets. The ''Pathway analysis methods'' section given next highlights our attempt to synthesize the recent and diverse genetic studies in the ARDS literature. A list of 201 mapped genes were identified *via* PMC/PubMed literature search of ARDS, acute lung injury (ALI), and ''lung injury'' studies that identified genes that were differentially expressed or conferred risk for ARDS, severe sepsis, or mortality (15) (Fig. 4 and Supplementary Table S1).

ARDS genes identified by dysregulated gene expression

The 201 ARDS genes were derived from studies with clinical populations, human-derived cell lines, or genes validated across multiple animal models with genetically conserved regions (29, 53, 82, 83, 149, 196). Cross-species analyses of VILI models (rat, mouse, canine) and human ARDS patients have yielded a list of genes that are conserved across species and of potential importance in the pathophysiology of ARDS and VILI (196). Eleven genes were ''immune response'' genes that were highly significant with Expression Analysis Systematic Explorer scores, and two genes (*IL-1B*, *IL-6*) were noted to harbor SNPs that were independently associated with ARDS risk or ARDS mortality (17, 180, 196).

Six genes were involved in ''inflammatory response'' and ''innate immune response'' pathways. Taken together, these data indicate that multiple genes fall into the evolutionary conserved inflammatory and immunological-related pathways across species and are potentially important in ARDS and VILI pathology (196). A limited mRNA study of ARDS and healthy controls yielded 12 upregulated genes in ARDS (106) with *IL-1R2*, a decoy receptor that dampens IL-1 signaling (106), identified as the top upregulated gene. Three genes (*Arginase-1*, *MHC-DRB1*, *CCR2*) are macrophage-specific genes expressed by activated macrophages (106).

Top Cited ARDS Genes	Acute Respiratory Distress Syndrome (ARDS) Citations	Acute Lung Injury (ALI) Citations	Sepsis/ Severe Sepsis Citations
JUN	3478	5808	15004
TNF	1126	3242	6231
ACE	137	201	93
SP-B	167	170	211
C3	144	156	473
VEGFA	122	265	138
CAT	94	322	706
IL6	74	158	332
EST	81	66	103
CCL2	80	230	235
HMGB1	72	339	726
C4	72	119	210
VWF	48	91	99

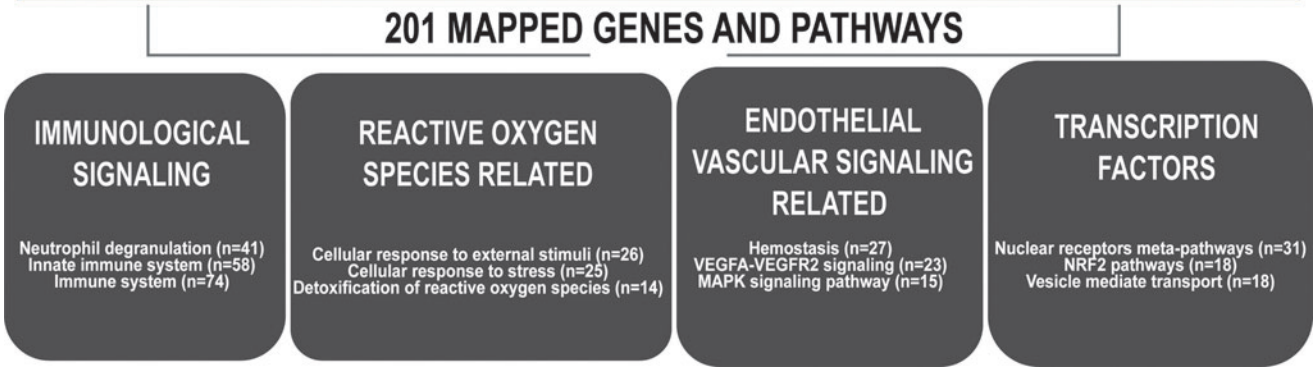

FIG. 4. ARDS gene table and top pathways. Two hundred one genes identified through differential gene expression (mRNA sequencing, mRNA CHIP array, RNA microarray, proteomics, candidate gene sequencing, or GWAS) that mapped onto either the Reactome or Wikipathways databases for pathway analysis (5359 human pathways covered). The second, third and fourth *columns* reflect the number of citations for each gene (14). Four broad categories of enriched pathways (immunological signaling, ROS-related signaling, vascular signaling, and transcription factor-related pathways) and the number or genes represented in each ($p<0.01$, five members minimum per pathway). GWAS, genome-wide association study; ROS, reactive oxygen species.

Another strategy to study pathways incorporating genes of interest is to utilize genetically engineered preclinical murine models involving exposure to ARDS and VILI followed by genome-wide lung tissue gene expression and pathway analysis (90). For example, *NAMPT*, also known as PBEF, is an ARDS candidate gene (175) that harbors several promoter SNPs (–2422A/G, –948G/T) that are associated with an increased risk of ARDS and ARDS mortality (3, 12, 108, 135, 169).

We have shown that extracellular NAMPT (eNAMPT) directly interacts with TLR4 (62) and genomic comparisons of wild-type mice and *NAMPT* heterozygous mice exposed to eNAMPT, VILI, or lipopolysaccharide revealed significant *NAMPT*-influenced pathways involved in ''acute phase response signaling,'' ''IL-10 signaling,'' ''IL-6 signaling,'' ''NF-κB signaling,'' ''LXR/RXR activation,'' ''Leukocyte Extravasation Signaling,'' ''PPAR signaling,'' ''Death Receptor Signaling,'' ''Apoptosis Signaling,'' and ''TLR signaling'' (35, 202). Similar genomic-intensive studies independently identified *TLR1* and interleukin-1 receptor-associated kinase (*IRAK1*) as ARDS risk genes (142, 155).

A complementary approach to identify pathways relevant to ARDS mortality is to utilize proteomic analyses to identify ARDS biomarkers that identify ARDS sub-phenotypes (21). Proteomic analysis of bronchoalveolar lavage fluid (BALF) in ARDS survivors and non-survivors (22) revealed differentially expressed proteins that fall within ''acute phase signaling'' and ''FXR/RXR Activation'' pathways, results remarkably similar to results from preclinical models of ARDS (22, 90).

The ''oxidative ethanol degradation'' and ''fatty acid α-oxidation'' pathways were significantly upregulated in BALF obtained from ARDS non-survivors (22). Utilization of the quantitative electrophoresis-based proteomics method (difference gel electrophoresis) identified 37 proteins differentially expressed between ARDS patients and healthy controls (39), with ''Wounding'' and ''Inflammatory Response'' being the top network pathways that included calgranulin A (*S100A8*), calgranulin B (*S100A9*), calgranulin C (*S100A12*), serum amyloid protein (*SAA*), complement C9 precursor (*C9*), hemopexin precursor (*HPX*), peroxiredoxin 5 mitochondrial (*PDX5*), complement C3 precursor (*C3*), annexin A1 (*ANXA1*), and alpha-1-antitrypsin (*SERPINA1*) (39).

PBMC gene expression in ARDS as predictors of mortality

PBMCs are an easily obtainable blood cell fraction that is broadly representative of innate immunity status. A meta-analysis of PBMC molecular biomarkers (54 distinct studies) attempted to validate ARDS gene biomarkers (175) and found two significant sets of biomarkers (175). One set consisted of ARDS risk genes and included Krebs von den Lugen-6 (*KL-6*), lactate dehydrogenase (*LDH*), soluble RAGE, and von Willebrand factor (*vWF*). A second set of genes associated with increased ARDS mortality (175) included *IL-4*, *IL-2*, angiopoetin 2 (*Ang-2*), and *KL-6*. *IL-4* is also an ARDS candidate gene with SNPs associated with ARDS risk, possibly *via* regulation of lung repair in cellular and animal models of ARDS (52, 85, 120, 173).

To further determine the utility of a PBMC-derived gene signature in the ICU setting, we interrogated differentially expressed PBMC genes in 55 ARDS survivors and non-survivors (Affymetrix GeneChip Human Exon 2.0 ST microarray). Figure 5A depicts heatmap-displayed results of bioinformatic analysis with 33 differentially expressed genes (DEGs) identified in a molecular signature in ARDS patients ($n = 23$) *versus* controls ($n = 80$), 19 genes downregulated, 14 upregulated (fold change $>2 \pm 4.41$, $p < 7.26e^{-23}$). Importantly, of the 215 genes (23 upregulated, 192 downregulated) predictive of survival with ''Toll-like receptor signaling pathway,'' the top enriched pathway, Figure 5B depicts DEGs in PBMCs from 23 ARDS patients that reflect survival with 16 downregulated genes and 5 upregulated genes (Fig. 5).

These gene lists includes genes previously reported as dysregulated in other ARDS studies (*PLAUR*, *IL1B*, *VEGFA*) (17, 83, 159). In addition, *IL1R2* harbors SNPs that confer increased risk for ARDS and represents a biomarker potentially predictive of sepsis-induced ARDS mortality (123). The gene with the greatest magnitude of upregulation was matrix metallopeptidase 8 (*MMP8*) with >400-fold change. Although levels of plasma proteins reflecting the expression of these genes were previously reported, this is the first article of MMP8 and TIMP-1 as genomic markers among non-survivors in ARDS (91). This link between MMP8 and IL1B as molecular biomarkers in blood and gene expression biomarkers predicting survival in ARDS suggests that this approach may yield clinically- and biologically relevant ARDS biomarker candidates (14, 58, 85, 91, 109, 120).

Figure 5B results are also consistent with other reports that the *TLR4* signaling pathway is a top pathway in predicting survival in ARDS patients (35, 92). eNAMPT, a validated ARDS blood biomarker whose *NAMPT* promoter SNPs confers risk of ARDS and ARDS mortality (3, 12, 108, 135, 169), is a TLR4 ligand (35) and both TLR4 and NAMPT are differentially expressed in animal models of ARDS (63, 173, 196). There is increasing interest in potentially therapeutically targeting the NAMPT pathway as a strategy to reduce ARDS mortality (Oita *et al.*, manuscript in preparation). In addition, *NAMPT* genotypes and plasma protein levels represent an opportunity to develop a panel of biomarkers/genotypes that could be employed for clinical trial stratification based on ARDS mortality risk (27, 105).

Pathway analysis methods

High-throughput screenings have provided a wealth of valuable genome-wide data with pathway analysis; the logical next step will be to integrate these results to understand the biological phenomena that underpin these data and generate future hypotheses (29, 100). Database analysis is currently coding protein focused, and multiple database searches can be used to integrate GWAS, mRNA, and proteomic studies (100). We employed multiple genomic and biological pathway database searches to compile the results of ARDS genomic studies to facilitate a better understanding of the pathways involved in a complex genetic disease such as ARDS.

Although pathway analysis is not a meta-analysis of ARDS patients, pathway analysis using multiple databases allows the results of candidate gene, agnostic GWAS studies, and unclassified studies to be combined for analysis and for a better understanding of the cellular and molecular pathways that are involved in ARDS pathobiology. Pathway analysis has two major benefits: (i) It allows for thousands of genes to

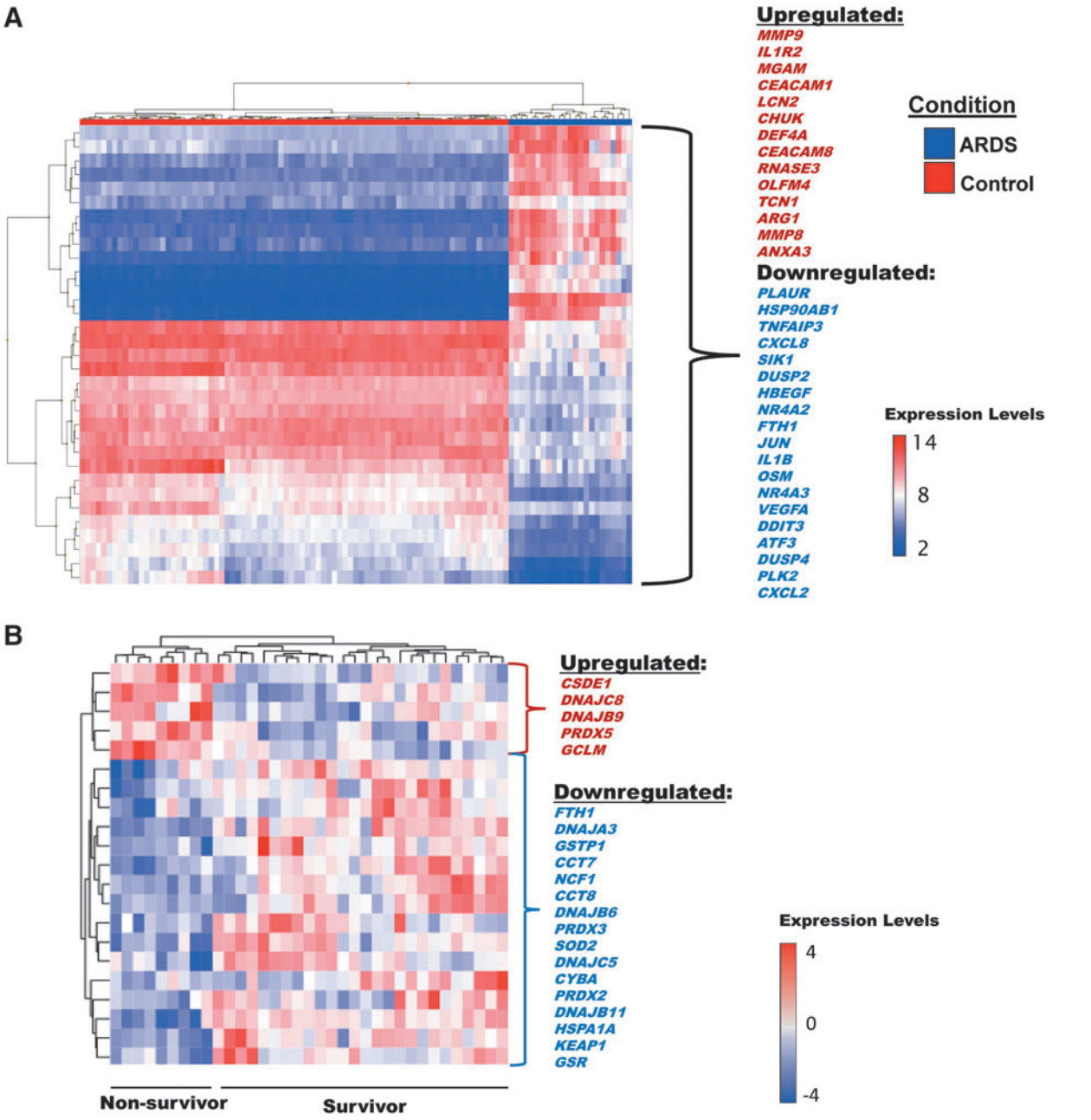

FIG. 5. Heatmap of PBMC gene expression predicting ARDS susceptibility and ARDS mortality. (A) The 33 top genes identified in a molecular survival signature from PBMC mRNA in ARDS patients ($n = 23$) *versus* controls ($n = 80$, PMID:19222302). Nineteen genes are downregulated, and 14 are upregulated (Fold change $>2 \pm 4.41$, $p < 7.26\text{e-}23$). **(B)** 22 genes in the ROS pathway were predictive of ARDS survival in ARDS patients. Five of these genes are upregulated, and 17 are downregulated (24). PBMC, peripheral blood mononuclear cell.

be reduced in complexity (29), and (ii) it allows for the development of active pathways that are significantly dysregulated in ARDS, potentially providing mechanistic insights that extend beyond creation of a simple gene list (29).

With a complex genetic disease such as ARDS, many genes with varying effect sizes will presumably be involved in ARDS pathobiology. Pathway analysis organizes the results of GWAS studies, candidate gene studies, and meta-analysis to understand the biological pathways that contribute significantly to disease progression. We used pathway analysis for gene sets (Gene Ontology terms), protein–protein interactions, and gene interactions (Reactome and Wikipathways; 5259 human pathways searched) to analyze our collected pool of 201 ARDS genes (100). These studies were performed on the Max Planck Institute for Molecular Genetics consensus pathway database (CPDB) across three pathway sources with the most relevance (Reactome and Wikipathways) (Table 1) (88, 102, 103).

Enriched pathways were defined as containing more than five genes represented and a p-value of <0.01, which resulted in 72 total enriched pathways. In addition, 38 relevant and/or highly enriched pathways were chosen based on clustering of

Table 1. Consensus Pathway Data Base Analysis: Thirty-Eight Enriched Acute Respiratory Distress Syndrome Pathways

Pathway name	*Candidates contained*	p-*Value*	Q-*Value*	*Pathway source*	*Genes contained*
Immunological pathways					
Immune system	74 (4.1%)	7.69e-17	1.29e-15	Reactome	ACTG1, AGER, ARG1, ARPC4, B2M, C3, C9, CAP1, CAT, CCT8, CEACAM1, CEACAM8, CHIT1, COTL1, CRISP3, CSF2, CSF2RB, CYBA, DEFA4, DNAJC5, FABP5, FGA, FTH1, FTL, GBP2, GHR, GPI, GSTP1, HBB, HMGB1, HSP90AB1, HSPA1A, HSPA8, IL13, IL1B, IL1R2, IL1RN, IL4, IL6, ISG15, JUN, KEAP1, LCN2, LGALS3, MAP3K1, MIF, MMP8, MMP9, MUC5AC, NCF1, NOS3, OLFM4, OSM, PDIA3, PGAM1, PI3, PLAUR, PPIA, PRDX6, RNASE3, S100A12, S100A8, S100A9, SAA1, SERPINA1, TCN1, TLR1, TNF, TNFAIP3, TNFRSF11A, TTR, TXN, VASP, YWHAZ
Innate immune system	58 (5.4%)	1.05e-18	5.29e-17	Reactome	ACTG1, AGER, ARG1, ARPC4, B2M, C3, C9, CAP1, CAT, CCT8, CEACAM1, CEACAM8, CHIT1, COTL1, CRISP3, CYBA, DEFA4, DNAJC5, FABP5, FGA, FTH1, FTL, GPI, GSTP1, HBB, HMGB1, HSP90AB1, HSPA1A, HSPA8, IL1B, ISG15, JUN, LCN2, LGALS3, MAP3K1, MIF, MMP8, MMP9, MUC5AC, NCF1, NOS3, OLFM4, PGAM1, PI3, PLAUR, PPIA, PRDX6, RNASE3, S100A12, S100A8, S100A9, SAA1, SERPINA1, TCN1, TLR1, TNFAIP3, TTR, TXN
Neutrophil degranulation	41 (8.5%)	7.87e-20	7.94e-18	Reactome	ARG1, B2M, C3, CAP1, CAT, CCT8, CEACAM1, CEACAM8, CHIT1, COTL1, CRISP3, CYBA, DEFA4, DNAJC5, FABP5, FTH1, FTL, GPI, GSTP1, HBB, HMGB1, HSP90AB1, HSPA1A, HSPA8, LCN2, LGALS3, MIF, MMP8, MMP9, OLFM4, PGAM1, PLAUR, PPIA, PRDX6, RNASE3, S100A12, S100A8, S100A9, SERPINA1, TCN1, TTR
Cytokine signaling in the immune system	21 (4.6%)	5.64e-06	1.73e-05	Reactome	AGER, B2M, CSF2, CSF2RB, GBP2, GHR, HMGB1, IL13, IL1B, IL1R2, IL1RN, IL4, IL6, ISG15, JUN, OSM, S100A12, SAA1, TNF, TNFRSF11A, YWHAZ
Signaling by interleukins	15 (5.9%)	6.95e-06	2.06e-05	Reactome	AGER, CSF2, CSF2RB, HMGB1, IL13, IL1B, IL1R2, IL1RN, IL4, IL6, JUN, OSM, S100A12, SAA1, YWHAZ
Interleukin-4 and 13 signaling	15 (15.3%)	1.67e-11	1.3e-10	Wikipathways	ANXA1, CCL2, CDKN1A, CEBPD, CXCL8, HSPA8, IL1B, IL6, LCN2, MMP9, OSM, PTGS2, S1PR1, SAA1, TNF
Interleukin 10 signaling	10 (26.3%)	1.57e-10	1.06e-09	Wikipathways	CCL2, CSF2, CXCL2, CXCL8, IL1B, IL1R2, IL1RN, IL6, PTGS2, TNF
Interleukin 1 signaling	7 (11.7%)	3.12e-05	7.32e-05	Reactome	AGER, HMGB1, IL1B, IL1R2, IL1RN, S100A12, SAA1
Development and heterogeneity of the ILC family	7 (21.9%)	4.02e-07	2.51e-06	Wikipathways	AHR, AREG, IL13, IL1B, IL4, IL6, TNF
Reactive oxygen species (ROS)-related pathways					
Cellular response to external stimuli	26 (6.3%)	6.14e-10	3.65e-09	Reactome	CAT, CDKN1A, CEBPB, CXCL8, CYBA, CYCS, DNAJA1, DNAJB6, EPAS1, GSR, GSTP1, HSP90AB1, HSPA1A, HSPA8, IL6, JUN, NCF1, NOX4, PRDX2, PRDX3, PRDX5, PRDX6, PRKAG2, SOD2, SOD3, TXN

(*continued*)

Table 1. (Continued)

Pathway name	*Candidates contained*	p-*Value*	Q-*Value*	*Pathway source*	*Genes contained*
Cellular responses to stress	25 (7.3%)	6.59e-11	4.75e-10	Reactome	CAT, CDKN1A, CEBPB, CXCL8, CYBA, CYCS, DNAJA1, DNAJB6, EPAS1, GSR, GSTP1, HSP90AB1, HSPA1A, HSPA8, IL6, JUN, NCF1, NOX4, PRDX2, PRDX3, PRDX5, PRDX6, SOD2, SOD3, TXN
Detoxification of reactive oxygen species	14 (38.9%)	5.52e-17	1.29e-15	Reactome	CAT, CYBA, CYCS, GSR, GSTP1, NCF1, NOX4, PRDX2, PRDX3, PRDX5, PRDX6, SOD2, SOD3, TXN
Focal adhesion	13 (6.3%)	1.51e-05	4.01e-05	Wikipathways	ACTG1, COL1A1, COL3A1, COL5A1, EGF, EGFR, FLNA, FLT1, ITGA1, JUN, MYLK, VASP, VWF
Toll-like receptor cascades	10 (6.5%)	0.000112	0.000242	Reactome	AGER, FGA, HMGB1, JUN, MAP3K1, S100A12, S100A8, S100A9, SAA1, TLR1
Oxidative stress	9 (30.0%)	3.63e-10	2.29e-09	Wikipathways	CAT, CYBA, CYP1A1, GCLC, GSR, NFE2L2, NOX4, SOD2, SOD3
Cardiovascular and signaling pathways					
Hemostasis	27 (4.0%)	2.58e-06	9.29e-06	Reactome	ANXA5, APOA1, CAP1, CEACAM1, CEACAM8, EGF, F3, FGA, FLNA, GYPA, GYPB, HRG, ITGA1, JMJD1C, MIF, NOS3, PFN1, PLAUR, PPIA, PRKAR2A, SELPLG, SERPINA1, SERPINE1, TGFB2, TMSB4X, VWF, YWHAZ
VEGFA-VEGFR2 signaling pathway	23 (9.7%)	1.01e-12	9.31e-12	Wikipathways	ANXA1, CCL2, CXCL8, DNAJB9, EZR, F3, FGA, GJA1, HBEGF, HSPA1A, JUN, MYH9, NCF1, NOS3, NR4A1, NR4A2, NR4A3, PFN1, PLAUR, PTGS2, SOD2, TXN
MAPK signaling pathway	15 (6.1%)	4.71e-06	1.59e-05	Wikipathways	EGF, EGFR, FLNA, GADD45A, HSPA1A, HSPA8, IL1B, IL1R2, JUN, MAP3K1, MAP3K6, NR4A1, STMN1, TGFB2, TNF
Platelet activation, signaling, and aggregation	15 (5.8%)	9.2e-06	2.58e-05	Reactome	ANXA5, APOA1, CAP1, EGF, FGA, FLNA, HRG, PFN1, PPIA, SERPINA1, SERPINE1, TGFB2, TMSB4X, VWF, YWHAZ
Platelet degranulation	14 (10.9%)	8.35e-09	4.68e-08	Reactome	ANXA5, APOA1, CAP1, EGF, FGA, FLNA, HRG, PFN1, PPIA, SERPINA1, SERPINE1, TGFB2, TMSB4X, VWF
Response to elevated platelet cytosolic Ca2+	14 (10.4%)	1.37e-08	7.28e-08	Reactome	ANXA5, APOA1, CAP1, EGF, FGA, FLNA, HRG, PFN1, PPIA, SERPINA1, SERPINE1, TGFB2, TMSB4X, VWF
Adipogenesis	13 (9.9%)	8.49e-08	4.08e-07	Wikipathways	ADIPOQ, AGT, AHR, CDKN1A, CEBPB, CEBPD, EPAS1, GADD45A, IL6, MIF, NAMPT, OSM, SERPINE1, TNF
Focal adhesion-PI3K-Akt-mTOR-signaling pathway	13 (4.3%)	0.000645	0.00113	Wikipathways	CDKN1A, COL1A1, COL3A1, COL5A1, EGF, EGFR, EPAS1, FLT1, GHR, HSP90AB1, NOS3, OSM, VWF
PI3K-Akt signaling pathway	13 (3.8%)	0.0019	0.00309	Wikipathways	CDKN1A, COL1A1, EGF, EGFR, FLT1, GHR, HSP90AB1, IL4, IL6, ITGA1, NOS3, OSM, VWF
AGE-RAGE	9 (13.6%)	5.88e-07	3.5e-06	Wikipathways	AGER, CYCS, EGFR, EZR, JUN, LGALS3, MMP9, NCF1, NOS3
Complement and coagulation cascades	7 (11.9%)	2.79e-05	7.04e-05	Wikipathways	C3, C9, F3, PLAUR, SERPINA1, SERPINE1, VWF
Transcription factor and signaling pathways					
Nuclear receptors meta-pathway	31 (9.8%)	6.42e-17	1.29e-15	Wikipathways	ABCB1, AGER, AHR, APOA1, CCL2, CYP1A1, EGFR, FTH1, FTL, GCLC, GCLM, GSR, GSTP1, HBEGF, HSP90AB1, HSPA1A, IL1B, JUN, KEAP1, NFE2L2, PDE4B, PLK2, PRDX6, PTGS2, SERPINA1, SOD3, TGFB2, TNF, TNFAIP3, TXN, UGT2B7

(*continued*)

Table 1. (Continued)

Pathway name	*Candidates contained*	p-*Value*	Q-*Value*	*Pathway source*	*Genes contained*
NFR2 pathway	18 (12.7%)	3.98e-12	3.35e-11	Wikipathways	AGER,FTH1, FTL, GCLC, GCLM, GSR, GSTP1, HBEGF, HSP90AB1, HSPA1A, KEAP1, NFE2L2, PRDX6, SERPINA1, SOD3, TGFB2, TXN, UGT2B7
Vesicle-mediated transport	18 (2.9%)	0.00588	0.00843	Reactome	AGTR1, APOA1, AREG, ARPC4, COL1A1, COL3A1, EGF, EGFR, FTH1, FTL, GJA1, HBB, HPX, HSPA8, PRKAG2, SAA1, SERPINA1, YWHAZ
G alpha (i) signaling events	16 (4.0%)	0.000347	0.000674	Reactome	AGT, ANXA1, APOA1, C3, CXCL2, CXCL8, CXCR4, GRM3, HSPG2, PDE4B, PRKAR2A, RBP4, S1PR1, S1PR3, SAA1, TTR
Transcriptional regulation by TP53	13 (3.5%)	0.00403	0.00598	Reactome	BTG2, CDKN1A, CYCS, GADD45A, GPI, GSR, JUN, PLK2, PRDX2, PRDX5, PRKAG2, TXN, YWHAZ
Photodynamic therapy-induced NF-kB survival signaling	8 (22.9%)	3.83e-08	1.93e-07	Wikipathways	CSF2, CXCL2, CXCL8, IL1B, IL6, MMP9, PTGS2, TNF
Other pathways					
Selenium micronutrient network	19 (22.9%)	1.1e-17	3.69e-16	Wikipathways	APOA1, CAT, CBS, CCL2, GSR, HBB, IL1B, IL6, MTHFR, PRDX2, PRDX3, PRDX5, PTGS2, SAA1, SERPINE1, SOD2, SOD3, TNF, TXN
Spinal cord injury	20 (17.1%)	6.47e-16	9.33e-15	Wikipathways	ANXA1, AQP1, ARG1, BTG2, CCL2, CXCL2, CXCL8, EGFR, GADD45A, GJA1, IL1B, IL4, IL6, LGALS3, MIF, MMP9, NOX4, NR4A1, PTGS2, TNF
Lung fibrosis	14 (21.9%)	4.82e-13	5.41e-12	Wikipathways	CCL2, CEBPB, CSF2, CXCL2, CXCL8, EGF, IL13, IL1B, IL4, IL6, MMP9, NFE2L2, SERPINA1, TNF
Folate metabolism	14 (21.2%)	7.59e-13	7.67e-12	Wikipathways	APOA1, CAT, CBS, CCL2, HBB, IL1B, IL4, IL6, MTHFR, SAA1, SERPINE1, SOD2, SOD3, TNF
Vitamin B12 metabolism	13 (25.5%)	3.99e-13	5.04e-12	Wikipathways	APOA1, CBS, CCL2, HBB, IL1B, IL6, MTHFR, SAA1, SERPINE1, SOD2, SOD3, TCN1, TNF
Sudden infant death syndrome (SIDS) susceptibility pathways	12 (7.5%)	5.04e-06	1.63e-05	Wikipathways	CEBPB, CXCL8, GJA1, HTR2A, IL13, IL1B, IL1RN, IL6, JUN, PRKAR2A, TNF, YWHAZ

HMGB1, high mobility group box 1; KEAP1, Kelch-like ECH associated protein 1; MIF, macrophage migration inhibitory factor; MYLK, myosin light chain kinase; NAMPT, nicotinamide phosphoribosyl transferase; *PRDXs*, peroxiredoxin gene family; S1Ps, sphingosine 1-phosphate receptors.

genes (Fig. 4; Table 1). Of the enriched pathways, 17 resided in Reactome and 21 in Wikipathways (Table 1) and were further divided into ROS pathways ($n = 6$), immune and inflammatory pathways ($n = 9$), cardiovascular signaling pathways ($n = 11$), transcription factor signaling pathways ($n = 6$), and other pathways ($n = 6$).

ROS pathways

ROS play an important role in sepsis and ARDS, and they contribute to the severe disruption of the endothelial barrier and the resulting inflammation and inflammatory cascade in the lower airway (54). Neutrophils migrate across the endothelial barrier in response to endothelial-secreted cytokines and chemoattractants. In addition to endothelial-secreted pro-inflammatory signaling, neutrophils are further sources of released pro-inflammatory cytokines, ROS, proteolytic enzymes, nitrogen species, cationic proteins, and lipid mediators (54). Each inflammatory cell type in the lung generates and releases distinct profiles of ROS molecules (45). Leukocytes express NADPH oxidase and nitric oxidase synthases, which together generate peroxynitrite and other ROS species (45).

In ARDS, polymorphonuclear neutrophils and macrophages initiate prolific ROS activation (45). Several isoforms of NADPH oxidase (*NOX1*, *NOX2*, *NOX4*, *NOX5*) are expressed in the endothelium, and increased expression of *NOX1*, *NOX2*, and *NOX4* drives endothelial and epithelial barrier dysfunction and generates substantial amounts of secondary ROS (19, 45, 81, 86). The main role of NOX is to catalyze the reduction of molecular oxygen (O_2) to superoxide (O_2^-) (19). Pulmonary endothelial cells express both *NOX2* and *NOX4* that generate ROS under hypoxic conditions as well on exposure to mechanical stress caused by VILI (81, 86).

ROS generation is linked to survival in sepsis patients (2, 11, 25, 69, 73, 74, 96, 99, 105, 148, 169, 197), and we recently reported that a 21-gene ROS gene signature was significantly linked to survival in sepsis (25) with ''Oxidative phosphorylation'' being the top enriched pathway. Oxidative phosphorylation is the major pathway of ATP generation in eukaryotic cells, including the vascular endothelium (139). Endothelial mitochondria are also a major source of ROS under aerobic conditions, which include encode complex organelles, including multiple peroxisomes (the P450 complex, xanthine oxidases, and nicotinamide adenine dinucleotide [NADPH] oxidase complexes) that are encoded by a large number of genes (139).

Mitochondria and many genes involved in ATP production create ROS byproducts. The list of ROS genes (Fig. 5B) include chaperon proteins (*HSP40*, *HSP70*), which were not commented on in the original paper (25) but are included in the Kyoto Encyclopedia of Genes and Genomes (KEGG) oxidative phosphorylation pathway, and these chaperon proteins are of potential mechanistic interest to understanding the role of oxidative phosphorylation in ARDS. The 21 ROS gene signature (*CSDE1*, *DNAJC8*, *DNAJB9*, *PRDX5*, *GCLM*, *FTH1*, *DNAJA3*, *GSTP1*, *CCT7*, *NCF1*, *CCT8*, *DNAJB6*, *PRDX3*, *SOD2*, *DNAJC5*, *CYBA*, *PRDX2*, *DNAJB11*, *HSPA1A*, *KEAP1*, *GSR*) was used to create a sepsis risk score (25) (Fig. 5B) and significantly outperformed a thousand randomly picked genes in predicting survival among ARDS patients (25).

In the wider ARDS literature, 27 other genes were identified in pathways related to oxidative and cellular stress (Table 1). NOX4 represented a link across multiple pathways (''detoxification of ROS,'' ''Cellular stress,'' ''Cellular response to external stimuli,'' and ''oxidative stress''). Both the superoxide dismutase (SOD) family (*SOD2*, *SOD3*) and the peroxiredoxin gene family (*PRDX2*, *PRDX3*, *PRDX5*, *PRDX6*) were also represented across multiple pathways (''Detoxification of ROS,'' ''Cellular stress,'' ''Cellular response to external stimuli'') (Table 1) and exhibited common variants associated with ARDS in case–control studies (87, 112). These genes, together with those that comprise the previously identified 21 gene signature (25), are relevant to a genetic ROS risk and survival signature in ARDS.

''Oxidative stress'' pathways join ''inflammation'' and ''apoptosis'' as pathways implicated as being important to ARDS pathology (22, 44, 90, 110, 151), with several ''Oxidative stress'' pathway genes exhibiting SNPs that are significantly linked to ARDS (*NAMPT*, *IL-6*, *IL4*, *IL-13*) and to vascular signaling pathways (44). Important redox-sensitive pathways in ARDS are the mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT) pathways that regulate several ARDS candidate genes (18, 135a, 182, 185, 203). Another redox-sensitive pathway involved in signaling and fibrotic proliferation is the PI3K/Akt pathway (36). Individually, many genes (*NOX1*, *NOX4*, *STAT4*, *STAT5*) in the MAPK/STAT and PI3K/Akt pathways exhibit mechanistic roles in ARDS pathology in animal models (36).

One highly ROS-related pathway is the NRF2 (*NFE2L2*) transcription factor and signaling pathway. NRF2 is a ubiquitous master transcription factor that regulates antioxidant response elements (*ARE*) and mediates cytoprotective and antioxidant protein expression (Fig. 5A) (43). In the healthy lung, NRF2 has a protective effect against hyperoxia, mechanical stress, and VILI (43, 150); it regulates NOX4 in the human and mouse lung, and ROS signaling in ARDS (141, 150).

NRF2 binds to Kelch-like ECH associated protein 1 (*KEAP1*), another differentially upregulated gene in the ROS ARDS survival gene signature (25, 150). The Keap1–Nrf2 complex translocates Nrf2 to the matrix that binds to AREs and transcribes heme oxygenase-1, NAD(P)H:quinone oxidoreductase 1, catalase, and SOD (41). Interestingly, NRF2 was recently shown to uniquely repress the expression of another ARDS candidate gene, *MYLK*, *via* a novel mechanism involving the AREs (113, 125, 141, 172), again highlighting the involvement of NRF2 in ARDS.

Immune-linked and inflammation-linked pathways

Many innate immunity genes are implicated in severe lung injury, contributing to neutrophil infiltration into the alveolar space and ''cytokine storm,'' an ARDS hallmark (90). In sepsis-induced ARDS, neutrophil-related genes (*OLFM4*, *CD24*, *LCN2*, *BPI*, *RBP7*, *UTS2*) are significantly expressed compared with sepsis patients alone (105). The most highly enriched pathways in our analysis were related to immune signaling (Table 1), with the caveat that many genes were shared between pathways.

A strong 37-gene signature (*ARG1*, *CAP1*, *CAT*, *CCT8*, *CEACAM1*, *CEACAM8*, *CHIT1*, *COTL1*, *CRIPS3*, *CYBA*, *DEFA4*, *DNAJC5*, *FABP5*, *FTH1*, *FTL*, *GPI*, *GSTP1*, *HBB*,

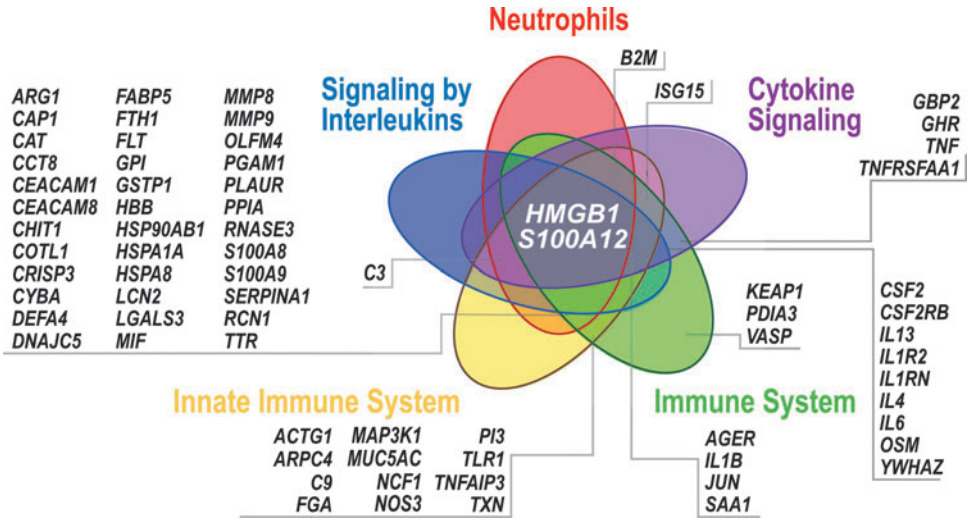

FIG. 6. Inflammation pathways. Using the entirety of 201 genes identified by our eGWAS approach, we identified the top five enriched inflammation pathways: neutrophils, cytokine signaling, immune system, innate immune system, leukocytes. Shown are the individual genes in each pathway with significant overlap. eGWAS, expression genome-wide association study.

HSP90AB1, *HSPA1A*, *HSPA8*, *LCN2*, *LGALS3*, *MIF*, *MMP8*, *MMP9*, *OFLM4*, *PGAM1*, *PLAUR*, *PPIA*, *RNASE3*, *S100A8*, *S100A9*) was shared between ''neutrophils,'' ''innate immune system,'' and ''immune system signaling'' pathways (Fig. 6). The strong neutrophil/immune system signature highlights dysregulation in neutrophil cellular biology as of key importance in ARDS risk and survival (84).

MMP8 and *MMP9*, two genes present in the ARDS survival signature depicted in Figure 6 (Manuel Gonzales-Garay, title unknown), are released by neutrophils at the site of acute inflammation (7). Both MMP8 and MMP9 had increased protein levels in models of lung injury (6, 7, 91). Absence of *MMP8* and *MMP9* in MMP8$^{-/-}$ and MMP9$^{-/-}$ mice exposed to VILI models shows decreased risk for ALI (6, 7). Both MMP levels in BALF correlate with increased lung injury (84).

HMGB1 is represented across all five of the top immune system pathways and in the IL-1 signaling pathway (Table 1; Figs. 6 and 7). *HMGB1* was identified as a cytokine in a murine model of endotoxin-mediated lethality (184) and is upregulated *in vitro* under 18% cyclic stretch conditions of high mechanical stress (194). Similar to eNAMPT, *HMGB1* binds *TLR4* as well as *RAGE*, the primary receptor of HMGB1 (133, 161). HMGB1 protein expression correlates with ARDS severity, 28-day mortality, and 90-day mortality (34, 94–96, 179).

Another important immunological cytokine in ARDS is MIF, first described in animal models of ARDS (20) and as a product of activated T cells that inhibit macrophage migration (71). In humans, MIF levels in BAL were elevated in both ARDS and septic patients, and we identified two SNPs (rs755622 and rs2070767) in MIF to be associated with African American ARDS patients (71). In pathway analysis, MIF is present in three of the immune pathways (neutrophils, immune system signaling, innate immune system signaling), hemostasis, and adipogenesis (Table 1).

IL-6 and IL-4 are well established biomarkers in ARDS (14, 25, 44, 79, 120, 175), and enrichment of IL signaling pathways in ARDS merits commentary (Fig. 7). Pathways for ''IL-4 and IL-13 signaling,'' ''IL-1B signaling,'' ''IL-10

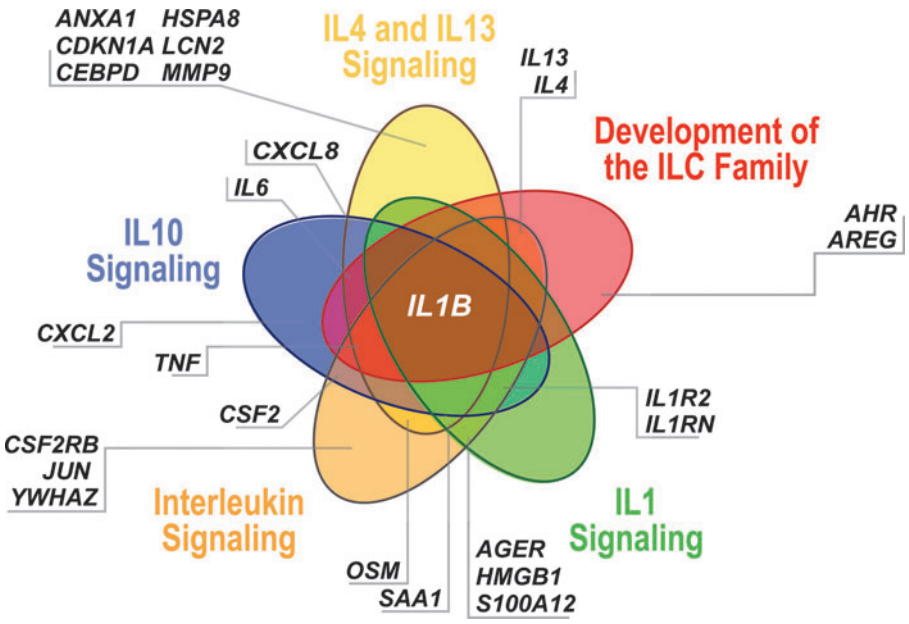

FIG. 7. Five interleukin-associated signaling-specific pathways. (IL-10 signaling, IL-4 and IL-13 signaling, development of the ILC family, IL-1 signaling, leukocytes) and the genes that overlap. IL, interleukin.

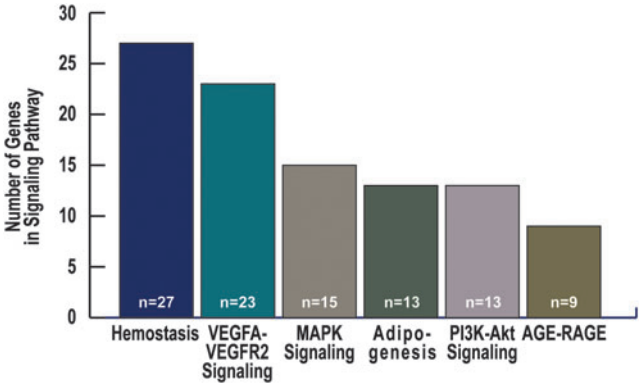

FIG. 8. Genes represented in top enriched endothelial vascular pathways. Shown are the top six cardiovascular signaling pathways and the number of genes in each pathway: Hemostasis, VEGFA-VEGFR2, MAPK signaling, PI3K-Akt signaling, AGE-RAGE. RAGE, receptor of advanced glycation end products; MAPK, mitogen-activated protein kinase; VEGF, vascular endothelial growth factor.

signaling,'' development of the ''ILC'' family, and ''leukocytes (general)'' were enriched in pathway analysis (Table 1). *IL1B* is involved in all pathways, and IL-1B is an early candidate biomarker whose serum levels correlate with endothelial cellular injury (120, 143).

eNAMPT-driven gene pathways after TLR4 ligation include ''IL10-signaling,'' ''IL-6 signaling,'' ''leukocyte extravasation signaling,'' and ''toll-like receptor signaling'' (90). A total of 28 genes are involved in at least one IL-related signaling pathway (*AGER*, *AHR*, *ANXA1*, *AREG*, *CCL2*, *CDKN1A*, *CEBPD*, *CSF2*, *CSF2RB*, *CXCL2*, *CXCL8*, *HMGB1*, *HSPA8*, *IL13*, *IL1B*, *IL1R2*, *IL1RN*, *IL4*, *IL6*, *JUN*, *LCN2*, *MMP9*, *OSM*, *PTGS2*, *S100A12*, *SAA1*, *TNF*, *YWHAZ*) (Table 1; Fig. 7).

Endothelial vascular and cellular signaling pathways

One of the most studied and diverse groups of genes responsible for the pathology of ARDS are the highly conserved vascular signaling genes (196). Our ARDS pathway analysis identified 11 pathways involved with vascular biology relevant to ARDS, with the 6 most highly enriched vascular and cellular signaling pathways shown in Table 1 and Figure 8 and genes from platelet-specific pathways shown in Figure 9. Both growth factors and coagulation factors are upregulated in mRNA studies across species (mouse, rat, canine, human) (196). Of the 37 genes upregulated in this cross-species study, 5 were related to cell proliferation, 6 were related to wound healing, 5 were related to extracellular spaces, and all were related to pro-fibrinolytic processes associated with poor outcomes in ARDS (167, 196). One of these genes, *SERPINE1*, encodes PAI-1, a potential biomarker for ARDS (137, 196).

Signaling by VEGF (KEGG pathway map04370) is associated with ARDS in a large genomic ARDS study that yielded 44 significant genes of interest (87), with 13 being specifically involved in VEGF signaling, a critical pathway for cellular proliferation in vascular signaling and ALI (87). Expression of the RAGE is correlated with severity in ARDS patients (97). RAGE is predominantly expressed in epithelial cells, and several *RAGE* SNPs are potential ARDS risk SNPs (30). *NOS3*, *IL-1B*, *NOX4*, *SERPINE1*, and *IL6* all have a history of associations with ARDS pathology, risk, and severity (17, 24, 86). The VEGF signaling pathway also triggers the downstream activation of many transcription factors such as SP1, which regulates the key cytoskeleton protein, non-muscle myosin light chain kinase (160).

Genes associated with platelet count and coagulation have been discovered to be indirect mediators of endothelial damage in ARDS (193). Five genes associated with platelet counts (*BAD*, *LRRC16A*, *CD36*, *JMJD1C*, *SLMO2*) in a meta-analysis were studied in a larger population of ARDS and at-risk controls (146, 192, 193). Five pathways (''hemostasis,'' ''platelet activation,'' ''signaling and aggregation,'' ''platelet degranulation, response to elevated platelet cytosolic Ca^{2+},'' ''complement and coagulation cascades'') were involved with platelet signaling or coagulation (Table 1; Fig. 9). In a study with a canine model of lung injury, 7.4% of the differentially regulated genes were in blood coagulation pathways (163).

CPDB pathway analysis identified four separate significant enrichment pathways involving platelets and coagulation (''Hemostasis,'' ''Platelet activation, signaling and aggregation,'' ''Platelet degranulation,'' ''Response to elevated platelet cytosolic Ca^{2+}'') (Table 1; Fig. 9). These pathways share 14 common genes that drive this platelet and coagulation pathway signal (Fig. 9). Many of these coagulation genes (*ANXA1*, *APOA1*, *FGA*, *PPIA*, *SERPINA1*) were identified in ARDS proteomic studies as well (39). Genes involved in the sphingolipid generation and signaling pathway, such as *S1PR1* and *S1PR3*, are highly abundant in platelets and are also potential novel biomarkers and risk SNPs (132, 155, 169, 170).

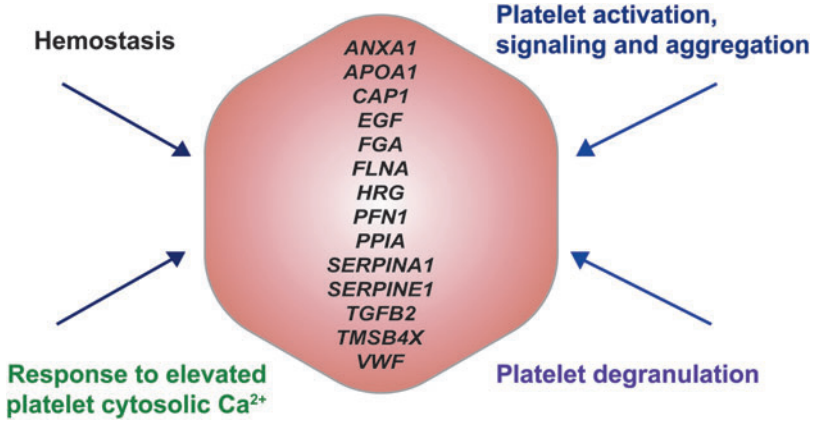

FIG. 9. Platelet and coagulation pathways. Fourteen genes are shared among the top four enriched platelet and coagulation pathways.

Other transcription factor and signaling pathways

Several important transcription factor and signaling pathways emerged from our pathway analysis (Table 1). Nuclear receptors can directly interact with DNA as a ligand, and the ''Nuclear receptors meta-pathways'' represent a diverse group of genes that point to the overall importance of DNA regulation and transcription in ARDS. Nuclear receptor meta-pathways are a nebulous category but they present a large and highly significant pathway identified in this study (Table 1; $p = 6.42e\text{-}17$). Of the 201 mapped ARDS genes, 31 represent either nuclear receptors or their interacting genes (Table 1) (*ABCB1*, *AGER*, *AHR*, *APOA1*, *CCL2*, *CYP1A1*, *EGFR*, *FTH1*, *FTL*, *GCLC*, *GCLM*, *GSR*, *GSTP1*, *HBEGF*, *HSP90AB1*, *HSPA1A*, *IL1B*, *JUN*, *KEAP1*, *NFE2L2*, *PDE4B*, *FLK2*, *PRDX6*, *PTGS2*, *SERPINA1*, *SOD3*, *TGFB2*, *TNF*, *TNFAIP3*, *TXN*, *UGT2B7*).

Critical Issues

ARDS genetic variants/genes identified by GWAS

The recent advances (149) in identifying risk SNPs for ARDS not only present new therapeutic opportunities for ARDS therapies but also present challenges for validation and replication across multiple cohorts in a heterogeneous genetic disease such as ARDS (122, 175). Attempts to address the challenge of defining genetic risk factors involved in the development of ARDS and the severity of the ARDS phenotype largely relies on two approaches: candidate gene studies and genome-wide association studies (149). Candidate gene studies focus on specific gene(s) with probable biological and mechanistic links to vascular permeability, cytoskeletal protein dysregulation, apoptosis pathways, or pro-inflammatory cascades (185, 203).

GWAS focus on genotyping the entire genome without requiring an *a priori* hypothesis regarding specific genes or their biological significance (5). GWAS studies benefit from not requiring an understanding of mechanisms of gene involvement, allowing for the discovery of novel genes in ARDS (149). Candidate gene studies have several limitations that GWAS studies overcome but have the potential advantage of facilitating defining SNP functionality. In complex diseases such as ARDS with a myriad of environmental and genetic causes, GWAS studies are valuable as they may be performed without a complete mechanistic understanding of the many biological pathways involved (5), with this agnostic approach allowing for the discovery of unique genotype–phenotype relationships (149).

GWAS studies exploring ARDS risk are primarily divided between European populations (80%) and African populations (20%) (24, 116, 123, 162, 173). Together, 5 GWAS studies have yielded 11 genes with 15 independent SNPs associated with ARDS susceptibility in GWAS case studies (Table 2).

Among European ARDS GWAS studies, nine ARDS genes, including XK-related 3 (*XKR3*), arylsulfatase D (*ARSD*), and Zinc-Finger/Leucine-Zipper Co-Transducer NIF1 (*ZNF335*), were identified (Table 2) by case–control whole exome sequencing of Asian American and European American populations (162). Another study in multiple ARDS populations (trauma- and sepsis-induced ARDS) of European descent identified several genes (*POPDC3*, *FAAH*, *PDE4B*, *ABCC1*, *TNFRSF11A*) to reach population-wide significance in a meta-analysis (173). Two other independent ARDS studies in European descent patients (Philadelphia, PA) had two genes (*IL1RN*, *PPFIA1*) reaching population-wide significance in their respective case–control studies (47, 123). *IL1RN* is linked to the development of both ARDS and sepsis (123, 140); *IL1RNA* levels were shown to be significantly higher in ARDS patients compared with controls, and predicted mortality (140).

Another immunity-related gene, *TNFRSF11A*, is a member of the tumor necrosis factor receptor (TNFR) family, mechanistically important to developing ARDS (78, 145). TNFR1 mediates cell death, inflammation, which, in turn, leads to vascular leak and neutrophil infiltration of the alveolar space (145). *TNFRSF11A* encodes the receptor activator of NF-kB (RANK), which is the receptor for receptor activator of NF-kB ligand (*RANKL*), key to altered NF-kB signaling (78, 165). Although *TNFRSF11A* SNPs have been significantly associated with the severity of Paget's disease, the reported ARDS risk SNPs are unique (78, 123) and may influence TNFRSF11A alternative splicing, a largely unexplored mechanism in ARDS (165, 181), and NF-kB signaling (78, 165).

GWAS studies of ARDS mortality

In the ARDS genetic literature, GWAS studies are less common than candidate gene studies due to their expense as well as that ARDS GWAS studies require larger patient populations to overcome the limitations of multiple association testing (Bonferroni correction) (149). Only two GWAS studies evaluated ARDS as an end-point with approximation of severity (11, 89, 117, 195), and both were conducted in European (89) or European descent populations (11, 116, 117). Each study had a single gene reach population significance for mortality association (*ADIPOQ*, *FER*, *ACE*) with at least one SNP, although other SNPs in linkage disequilibrium were reported (Table 2).

ADIPOQ encodes adiponectin with an SNP associated with the ARDS mortality study (4), and it has been linked in several meta-analyses to type 2 diabetes or obesity (114, 178) in Caucasians, in a South Eastern Asian population (114) and with type 2 diabetes in Chinese and Taiwanese populations (4, 48, 178, 195). However, after adjusting for body mass index, a measure of obesity, and for diabetes status, rs2082940 remained significantly associated with ARDS mortality (4).

Unlike *ADIPOQ*, *ACE* has a history of mechanistic studies that implicate ACE as an important gene in ARDS pathology (77, 198, 201). ACE is the enzyme that degrades angiotensin I (Ang I) to angiotensin II (Ang II), which is the peptide that is primarily responsible for maintaining blood pressure homeostasis and fluid/salt balance in kidney filtration (198). In animal models, ACE is strongly associated with elevations in IL-6 and leukocyte counts (201) and it is elevated in human ARDS BALF (198, 201).

Ang I has also been implicated as having an insertion/deletion polymorphism that is associated with mortality in an ICU ARDS cohort (116). *FER* is a member of the FPS/FES non-transmembrane receptor tyrosine kinase family, and it is significantly associated with ARDS (Table 2) and survival in septic patients (148). In a multi-cohort study, rs4957796 was significantly associated with survival in sepsis patients (148) and with increased survival in ARDS (89). Many ARDS

Table 2. Top Single Nucleotide Polymorphisms and Genes in Acute Respiratory Distress Syndrome Literature—Genome-Wide Association Study Risk

Gene	*Predictive SNPs*	*Population*	*Study*
GWAS risk SNPs			
ABCC1	Rs3887893 ($p=0.0001$, meta)	European descent (MGH, Boston, MA)	765 Stage II ARDS trauma population, 838 stage II ARDS sepsis population; direct *vs.* indirect ARDS association and meta-analysis (75)
ARSD	Rs78142040 (3.64e-47)	ARDSNet	213 ARDS patients, 440 (379 EUR and 61 ASW) controls; Exome-seq case/control association (161)
FAAH	Rs324420 ($p=0.0131$)	European descent (MGH, Boston, MA)	765 Stage II ARDS trauma population, 838 stage II ARDS sepsis population; direct *vs.* indirect ARDS association and meta-analysis (75)
HEATR1	Rs2115740($p=6.53\times10^{-5}$, unadjusted)	African American descent (Seattle, WA and Chicago, IL)	232 ARDS cases, 162 ICU controls (53)
IL1RN	Rs315952 ($p=0.0023$), rs380092 ($p=0.026$)	European descent (Philadelphia, PA)	Association stage II ($n=606$) ARDS and stage III ($n=561$) ARDS (74)
PDE4B	Rs12080701 ($p=0.0005$, meta), Rs17419964 ($p=0.0002$, meta)	European descent (MGH, Boston, MA)	765 Stage II ARDS trauma population, 838 stage II ARDS sepsis population; direct *vs.* indirect ARDS association and meta-analysis (75)
POPDC3	rs1190286 ($p=0.0094$)	European descent (MGH, Boston, MA)	765 Stage II ARDS trauma population, 838 stage II ARDS sepsis population; direct *vs.* indirect ARDS association and meta-analysis (75)
PPFIA1	Rs471931($p=0.0021$)	European descent (Philadelphia, PA)	Two-stage GWAS; phase 1 compared 600 ARDS trauma-associated ALI, 2266 population-based controls; phase 2 compared 212 ALI cases and 238 at-risk controls (71)
SELPLG	Rs109017898($p=1.5\times$e-04, $p=0.005$, discovery, meta)	African American descent (Seattle, WA and Chicago, IL)	232 ARDS cases, 162 ICU controls (53)
TACR2	Rs61732394($p=6.24\times10^{-4}$)	African American descent (Seattle, WA and Chicago, IL)	232 ARDS cases, 162 ICU controls (53)
TNFRSF11A	Rs9960450 ($p=5.3\times10^{-3}$, meta), Rs17069902 ($p=0.0001$, meta)	European descent (MGH, Boston, MA)	765 Stage II ARDS trauma population, 838 stage II ARDS sepsis population; direct *vs.* indirect ARDS association and meta-analysis (75)
XKR3	Rs9605146 (1.68×10^{-59})	ARDSNet	213 ARDS patients, 440 (379 EUR and 61 ASW) controls; Exome-seq case/control association (161)
ZNF335	Rs3848719 ($p=2.86$e-04)	ARDSNet	213 ARDS patients, 440 (379 EUR and 61 ASW) controls; Exome-seq case/control association (161)
GWAS survival-specific risk SNPs			
ADIPOQ	Rs2082940 ($p=0.0039$)	ICU (MGH and BIDMC, Boston)	2067 ICU patients; 567 ARDS patients; prospective risk and mortality study (169)
FER	Rs4957796 ($p=0.0144$)	European descent (Gottingen, Germany)	441 Total ARDS patients, 274 ARDS patients with pneumonia; 90-day survival; prospective case–control (166)

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ASW, African ancestry in the Southwest USA; EUR, European; GWAS, genome-wide association study; ICU, intensive care unit; SNP, single nucleotide polymorphism; XRK3, XK-related 3.

patients have sepsis as a comorbidity, and rs4957796 being an SNP associated with both mortality in ARDS and sepsis makes it a strong candidate gene to study in ARDS, and potentially useful in developing an ARDS risk SNP panel (89, 148).

ARDS genetic variants/genes identified by candidate gene studies

Candidate gene studies require an *a* priori hypothesis or prior knowledge of a gene's function, which make candidate gene studies popular for studying genes with known functional roles in ARDS (5). A limitation of candidate gene studies is a potential failure to account for genetic drift and population demographics on natural selection (5). In ARDS, this is a particularly important limitation because of observed health disparities between African, Hispanic, and European populations (64). The largest category of ARDS genetic studies are ARDS risk-association candidate gene studies (2, 12, 17, 24, 80, 93, 111, 154, 158, 174, 188). Candidate gene studies are the original genetic approaches in the ARDS literature and are often associated with a gene or protein's hypothesized role in lung injury (149).

The first reported candidate risk genes were *SFTPB* (*SP-B* variant), *ACE*, and *IL-6* (68, 80, 111, 116, 117). The advantage of candidate gene studies is that analyses can be performed in smaller population case sizes to achieve significance when compared with GWAS approaches (111, 116, 117). Although candidate gene studies are numerous, replication of specific SNPs has proven difficult. However larger study populations have produced more robust results for specific risk genes (*NFE2L2*, *NAMPT/PBEF*, *IL-4*, *IL-13*, *SP*-B, *AGER*, *PI3*, *MAP3K1*, *IL-6*, *MYLK*) and specific SNPs of interest (Table 2). Among candidate gene risk studies, 18 of 23 studies ($\sim$78%) have been performed exclusively in European populations or populations of European descent (11, 12, 24, 80, 93, 111, 154, 158, 174, 188).

The early research into candidate genes provided viable genotype–phenotype links for biomarker studies, and one of the most successful has been *IL-6* (117). A haplotype of *IL-6*, -174G/C, has been identified and validated as a risk SNP for several ARDS case–control studies (68, 118). Increased levels of IL-6 cause a rise in ROS *via* the *TLR4-TRIF-TRAF6* pathway (92). IL-6 is elevated in patients with ARDS and has been shown to have a significant role in the permeability of the lung endothelium in multiple ARDS mouse models (79). IL-6 has several risk SNPs and promoter haplotypes associated with sepsis-related ALI (66, 79). IL-6 levels are determined by many genetic factors, and the SNPs associated with ALI in sepsis patients were discovered in a Hispanic population (66).

Most importantly, two of the SNPs (–597G/–174G) are associated with a risk haplotype (118). A strong phenotype–genotype relationship with IL-6, genotypes, clinical outcomes, and ARDS severity or mortality has been documented, and the case for IL-6 genetics playing a role in ARDS severity risk and mortality is strong (66, 117, 118).

Candidate gene studies of ARDS mortality

Although there are more candidate gene studies that focus on ARDS risk genes, multiple candidate gene studies have evaluated ARDS mortality and associated risk genes (4, 12, 17, 61). When ARDS patients are stratified by the Berlin definition (mild, moderate, and severe), the more severe ARDS patients are significantly more likely to die from ARDS than patients with mild or moderate ARDS (16). Thus, patients who die from ARDS can be argued to have severe ARDS (16).

Of the four candidate gene studies that reported on mortality, three genes (75%) were obtained in European descent populations, and one population is from a pediatric, Brazilian population (Table 3). In the European populations, *NAMPT/PBEF*, *IL-1*β, and *PHD2* were identified as each having at least one SNP (*NAMPT/PBEF* has four) that is associated with ARDS mortality (4, 17, 61). In the Brazilian, pediatric ARDS population, *TNF* had two SNPS that were associated with death in septic and ARDS populations (4).

Two of these candidate genes (*TNF* and *IL-1*β) were chosen because they are molecules with a long history as ARDS biomarkers (11, 31, 120). IL-1β transcription is caused by stress and endotoxin triggers and is secreted by macrophages, thrombocytes, and injured endothelial cells (31, 55). The promoter for *IL-1*β includes NF-kB sites and activating protein-1 sites (55). In an attempt to link biomarkers to genotype and establish a genotype–phenotype relationship in IL-1β, a significant SNP was found in the *IL-1*β promoter region (-511 upstream from the TATA box and transcription start site) (17, 55). The site found to be related to ARDS and sepsis mortality was previously reported to be an important site for the secretion of IL-1β (143).

ARDS risk SNPs in African Americans

Racial and ethnic disparities in ARDS mortality and disease susceptibility have been reported (64); however, genetic studies in ARDS have focused on larger, European cohorts. Although this has provided a strong foundation for the understanding of ARDS genetics, population diversity among ARDS should not be discounted in the sub-phenotyping, diagnosis, and treatment of ARDS. Understanding genetic population diversity in ARDS is critical because there is a significant difference in mortality rates between European and African descent populations (49, 64).

Across multiple age populations (until the age of 65), African Americans have significantly higher rates of both sepsis and ARDS than their matched European American cohorts (16, 23, 49, 51, 129), greater duration on mechanical ventilation than European Americans (64), and a higher risk of ARDS mortality (16, 23, 129) when compared with age-matched European American counterparts. Hispanic Americans also have significantly higher mortality rates (5).

The health disparity borne by African Americans in ARDS and severe sepsis warrants additional research and attention to the role of genetics in identifying unique biomarkers and genetic markers for African Americans at risk for ARDS. Several unique candidate genes *MYLK*, *HEATR1*, *MIF*, *GADD45a*, *DIO2*, *SELPLG*, and *S1PR3* are promising genetic markers for increased risk of ARDS and ARDS mortality among African Americans (Fig. 10) (24, 46, 71–73, 115, 155, 169). In the case of *MYLK*, the gene encoding myosin light chain kinase, a risk haplotype was identified consisting of coding SNPs with one of these SNPs verified in other inflammatory disorders, including sickle cell disease and severe asthma in African Americans (67).

TABLE 3. TOP SINGLE NUCLEOTIDE POLYMORPHISMS AND GENES IN ACUTE RESPIRATORY DISTRESS SYNDROME LITERATURE—CANDIDATE GENE RISK

Gene	*Predictive SNPs*	*Population*	*Study*
Candidate gene risk SNPs			
ACE	D/D ($p = 0.00004$, healthy population, $p = 0.0008$ CABG patients)	European descent (University of College London Hospitals)	88 Respiratory failure patients, 174 CABG controls (192)
AGER	Rs2070600 (A/A, Ser/Ser, $p < 0.0001$)	European descent (Clermont-Ferrand, France)	59 ARDS, 405 controls; log rank test with case–control (178)
AGT	Rs699 (0.028, dom)	European descent (Moscow, Russia)	68 NP ARDS cases, 198 NP controls (179)
AhR	Rs2066853 (0.0012, dom)	European descent (Moscow, Russia)	68 NP ARDS cases, 198 NP controls (179)
CYP1A1	Rs2606345 (0.0027, dom)	European descent (Moscow, Russia)	68 NP ARDS cases, 198 NP controls (179)
DIO2	Rs12885300 ($p = 0.039$) Rs225014 ($p = 0.009$)	African and European descent (American-European Consensus Criteria)	327 European Americans: 139 sever sepsis, 78 severe sepsis + ARDS/ALI, 188 controls; 261 African Americans: 78 severe sepsis, 41 severe sepsis + ARDS/ALI, 187 controls (187)
EGF	Rs4444903 ($p = 0.005$, males), rs2298991 ($p = 0.019$, males), Rs7692976 ($p = 0.005$, males), Rs6533485 ($p = 0.025$, males)	European descent (MGH, Boston, MA)	416 ARDS cases (246 survivors, 170 died), 1052 ICU controls; 887 males and 581 females (180)
GADD45a	Rs581000 ($p = 0.009$)	African Americans Chicago Study and Spanish Study (Chicago, IL)	African American Chicago cohort: 71 severe sepsis, 40 sepsis + ALI, 182 controls; Spanishcohort: 80 severe sepsis, 66 sepsis + ALI, 95 controls (61)
IL-13	1 SNP (431 A>Gr, $p = 0.008$)	European descent (Moscow, Russia)	347 Controls, 74 ARDS cases; logistic regression adjusted case/control (98)
IL-4	1 SNP (−589 C>T, $p = 0.01$)	European descent (Moscow, Russia)	345 Controls, 72 ARDS cases; logistic regression adjusted case/control (98)
IL-6	−174G/C allele ($p = 0.03$)	European descent	Twin study on lung function, 427 twins (232 women, 195 men) (36, 41)
MAP3K1	Rs832582 ($p = 0.01$, rec)	FACTT and ARDSNet (Seattle WA)	241 ARDS patients, 346 healthy, locally matched controls (193)
MIF	2 SNPs (rs755622, $p = 0.03$; Rs2070767, $p = 0.04$)	European descent, African descent	288 European: 113 severe sepsis, 90 sepsis-associate ALI, 85 healthy controls; 218 African: 69 severe sepsis, 61 sepsis-associated ALI, 88 healthy controls (50)
MYLK	Rs820336 ($p = 0.002$) Rs936170 ($p = 0.009$) Rs936170 ($p = 0.025$)	European and African descent (John Hopkins University and Medical College of Wisconsin)	European: 92 ALI, 99 sepsis, 85 healthy controls; African: 43 ALI, 51 sepsis, 61 healthy control (91)
NAMPT/PBEF	2 SNPs (−948, $p = 0.015$–2422, $p = 0.03$)	European descent	374 ARDS patients and 787 at-risk controls; nested case–control (88)
NFE2L2	7 SNPS (p-values: 0.0069–0.0089)	Spanish Network	321 Severe sepsis and ARDS; 871 population-based controls; case–control (76)

(*continued*)

Table 3. (Continued)

Gene	*Predictive SNPs*	*Population*	*Study*
PI3	Rs1983649 ($p=0.034$, add), Rs2664581 ($p=0.004$, 0.023, add, dom)	European descent (MGH, Boston, MA)	449 ARDS patients, 1031 at-risk controls; case–control (181, 182)
S1PR3	2 SNPS (rs7022797, $p=0.017$; Rs11137480, $p=0.042$)	European and African descent (Chicago, IL)	71 European ARDS and 24 African ARDS; 186 European controls, 185 African controls (66)
SP-B	1 SNP (606-bp variant allele, 1580 C/T)	MGH (Boston, MA), European descent (German)	72 ARDS cases, 117 controls; nested case–control (177) 52 ARDS patients, 46 healthy controls (44)
TLR1	1 SNP (rs5743551, $p=0.002$)	European descent (Seattle, WA)	138 Severe sepsis ARDS, 107 ALI, 167 healthy controls (23)
Candidate gene survival-specific risk SNPs			
IL-1B	1 SNP (−511 G>A, $p=0.0019$)	European descent (Moscow, Russia)	321 Controls, 91 mortality cases; adjusted logistic regression for case–control mortality (150)
NAMPT/PBEF	−1001G ($p=0.001$) −1543T ($p=0.03$)	European descent	374 ARDS patients and 787 at-risk controls; nested case–control (101)
PHD2	1 SNP (rs516651, $p=0.002$)	European descent (Duisburg-Essen, Germany)	264 ARDS (70 died); case–control (184)
TNF	TNF-308 ($p=0.0006$) TNF-863 ($p=0.01$)	Brazilian septic and ARDS pediatric patients	490 Septic and ARDS patients; 610 controls (119)

CABG, coronary artery bypass graft; D/D, deletion/deletion; DIO2, iodothyronine deiodinase 2; *GADD45a*, growth arrest and DNA damage-inducible gene; NP, nosocomial pneumonia.

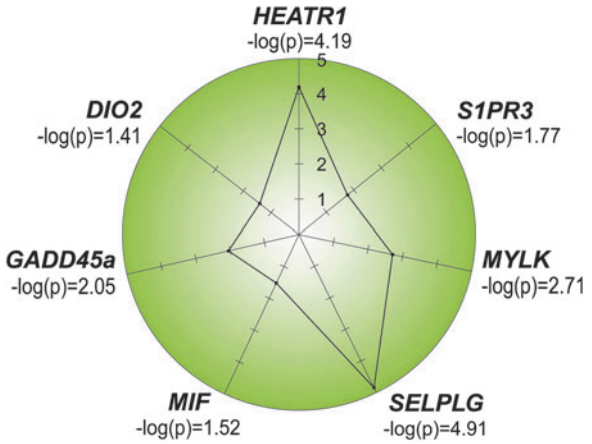

FIG. 10. Risk genes in African descent and Hispanic ethnicity. Depicted are the seven top risk single nucleotide polymorphisms/genes that are unique to ARDS risk in African descent individuals and to ARDS survival. The genes were identified *via* interrogation of GWAS results (*HEATR1*, *SELPLG*) or *via* candidate gene approaches and sequencing (*S1PR3*, *MYLK*, *DIO2*, *GADD45a*). *DIO2*, iodothyronine deiodinase 2; *GADD45a*, growth arrest and DNA damage-inducible gene; *MYLK*, myosin light chain kinase; S1Ps, sphingosine 1-phosphate receptors.

The functionality of the SNP has been shown to cause a delay in restoration of the vascular barrier in inflammatory models as well as to cause secondary mRNA structure alterations that promote excessive expression of this major cytoskeletal regulatory protein (67, 72, 73, 187, 204).

Our group recently conducted a GWAS study of African American ARDS patients and ICU controls that was underpowered but after innovative pathway prioritization, it discovered three novel genes that achieved genome-wide significance (24). Unlike in the European American ARDS studies, the population size in this study was relatively small ($n = 232$) (Tables 2 and 3; Fig. 10) (24). Two unique risk SNPs for ARDS (rs2115740, *HEATR1*; rs109017898, *SELPLG*) were found in this African American GWAS study in two genes that had not previously been identified as ARDS risk genes. Further, higher-powered GWAS studies in non-European populations may potentially provide more novel genes and SNPS that are risk factors for ARDS in other under-studied populations.

Future Directions

ARDS is a severe, high-mortality complex and heterogeneous critical illness influenced by environmental and genetic factors. In this review, we have collated the available preclinical and human ARDS literature and identified 201 pooled ARDS candidate genes (Supplementary Table S2) in a multi-database approach. Although we highlighted risk SNPs from both candidate gene studies and GWAS, pathway analysis allowed genes without known SNPs but reported mRNA and protein fold change to be included in our pathway analysis (25, 167, 196).

Our pathway analysis strategies revealed results that were consistent with the concept that evolutionarily conserved inflammatory and ROS networks and vascular gene dysregulation are potent contributors to ARDS pathobiology (82, 83). A broader ''omics'' approach to ARDS allows for the focus on biologically relevant pathways and genotype–phenotype connections between established ARDS biomarkers and differentially expressed ARDS risk genes.

We have also chosen to evaluate ARDS studies that use mortality as an end-point as this captures the most severe outcome for ARDS patients and summarized the evidence from genetic studies in diverse populations that have the potential to uncover novel biomarkers for ARDS risk and mortality and potential therapeutic targets in ARDS. We highlighted information relevant to the role of genetic factors in ARDS susceptibility and mortality (23) that address the well-known health disparities that exist in susceptibility to and mortality from ARDS (23, 38, 75). Improved strategies for sub-phenotyping of diverse ARDS patients *via* molecular signatures or SNP panels will facilitate the potential for successful clinical trials in ARDS and yield a better fundamental understanding of ARDS pathobiology (105).

Supplementary Material

Supplementary Table S1
Supplementary Table S2

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Abbreviations Used

ACE = angiotensin-converting enzyme
ALI = acute lung injury
ARDS = acute respiratory distress syndrome
ARE = antioxidant response elements
BALF = bronchoalveolar lavage fluid
CPDB = consensus pathway database
DEG = differentially expressed gene
DIO2 = iodothyronine deiodinase 2
eNAMPT = extracellular NAMPT
GWAS = genome-wide association study
HMGB1 = high mobility group box 1
ICU = intensive care unit
IL = interleukin
KEAP1 = Kelch-like ECH associated protein 1
KEGG = Kyoto Encyclopedia of Genes and Genomes
KL-6 = Krebs von den Lugen-6
MAPK = mitogen-activated protein kinase
MIF = macrophage migration inhibitory factor
MMP = matrix metallopeptidase
MYLK = myosin light chain kinase
NADPH = nicotinamide adenine dinucleotide
NAMPT = nicotinamide phosphoribosyl transferase
NOX = NADPH oxidase
PBMC = peripheral blood mononuclear cell
PRXs = peroxiredoxin gene family
RAGE = receptor of advanced glycation end products
ROS = reactive oxygen species
S1Ps = sphingosine 1-phosphate receptors
SNP = single nucleotide polymorphism
SOD = superoxide dismutase
STAT = signal transducer and activator of transcription
TLR4 = toll-like receptor 4
TNFR = tumor necrosis factor receptor
VEGF = vascular endothelial growth factor
VILI = ventilator-induced lung injury
XKR3 = XK-related 3