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Polymorphisms in key pulmonary inflammatory pathways and the development of acute respiratory distress syndrome

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Abstract

Purpose/Aim—Acute Respiratory Distress Syndrome (ARDS) is an important clinical and public health problem. Why some at-risk individuals develop ARDS and others do not is unclear but may be related to differences in inflammatory and cell signaling systems. The Receptor for Advanced Glycation Endproducts (RAGE) and Granulocyte-Monocyte Stimulating Factor (GM-CSF) pathways have recently been implicated in pulmonary pathophysiology; whether genetic variation within these pathways contributes to ARDS risk or outcome is unknown.

Materials and Methods—We studied 842 patients from three centers in Utah and 14 non-Utah ARDS Network centers. We studied patients at risk for ARDS and patients with ARDS to determine whether Single Nucleotide Polymorphisms (SNPs) in the RAGE and GM-CSF pathways were associated with development of ARDS. We studied 29 SNPs in 5 genes within the two pathways and controlled for age, sepsis as ARDS risk factor, and severity of illness, while targeting a false discovery rate of ≤5%. In a secondary analysis we evaluated associations with mortality.

Results—Of 842 patients, 690 had ARDS, and 152 were at-risk. Sepsis was the risk factor for ARDS in 250 (30%) patients. When controlling for age, APACHE III score, sepsis as risk factor, and multiple comparisons, no SNPs were significantly associated with ARDS. In a secondary analysis, only rs743564 in CSF2 approached significance with regard to mortality (OR 2.17, unadjusted $p = 0.005$, adjusted $p = 0.15$).

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Conclusions—Candidate SNPs within 5 genes in the RAGE and GM-CSF pathways were not significantly associated with development of ARDS in this multi-centric cohort.

Keywords

acute respiratory distress syndrome; GM-CSF; genetics; RAGE

INTRODUCTION

Acute Respiratory Distress Syndrome (ARDS), [1] is a serious clinical and public health burden [2]. Short-term mortality associated with ARDS is 20–40%, with more recent studies suggesting mortality nearer 20% [3–6]. ARDS arises from a complex, dysfunctional interplay among biological systems, involving immune/repair response, cytotoxicity and tissue injury, microvascular coagulopathy, and endothelial dysfunction. They are complicated by iatrogenic factors such as ventilator-associated lung injury and iatrogenic volume overload, among others [7]. Treatments that focused exclusively on the inflammatory response have failed to produce clinical benefits in ARDS, [2] or in sepsis [8], the most common risk factor for ARDS. Recent laboratory and clinical work have suggested that the multiligand pattern recognition Receptor for Advanced Glycation End-products (RAGE) and the Granulocyte-Monocyte Colony Stimulating Factor (GM-CSF, also known as CSF-2) pathways may be useful therapeutic targets in ARDS. These novel pathways both influence inflammation and epithelial cell signaling and function.

Based on considerations suggesting significant potential impact of these pathways on the pathobiology of ARDS, we evaluated whether single nucleotide polymorphisms (SNPs) within key genes identified in prior work as possible targets within the GM-CSF [9–11] or RAGE [12] pathways were associated with development of ARDS among at-risk patients.

Materials and Methods

Setting

At-risk patients were drawn from the ICUs at Intermountain Medical Center, a 450-bed tertiary-care, academic hospital in Murray, Utah; LDS Hospital, a 300-bed secondary-care, academic hospital in Salt Lake City, Utah; and the University of Utah Health Sciences Center, a 470-bed, tertiary-care, academic hospital in Salt Lake City, Utah. *Patients with ARDS* were drawn from the same Utah hospital ICUs and from patients at 14 non-Utah centers who participated in the NHLBI ARDS Network ALVEOLI [13], FACTT [14, 15], and ALTA [16] clinical trials.

Patients

We studied two basic patient populations: (1) Individuals *at-risk for ARDS who never met criteria for ARDS* and (2) *Patients with ARDS*. The at-risk patients came *exclusively* from Utah ICUs at Intermountain Medical Center, LDS Hospital, and the University of Utah Health Sciences Center. The ARDS population came from Utah ICUs and NHLBI ARDS Network patients.

Risk factors for ARDS included sepsis, pneumonia, multiple transfusions, significant trauma, or gastric acid aspiration, as described by Gong et al. [17, 18]. Specific criteria are displayed in eTable 1 of the online supplement. We enrolled at-risk patients within 48 hours of meeting at-risk criteria. The presence of a single risk factor classified a patient as at-risk. If at-risk patients subsequently met criteria for ARDS within 7 days of enrollment, they were removed from the at-risk group and reclassified as ARDS patients.

We used the NIH/NHLBI ARDS Network inclusion and exclusion criteria for ARDS patients at all centers. We defined ARDS by consensus criteria [19, 20], as displayed in eTable 2 of the online supplement. ARDS patients were enrolled within 48 hours of meeting consensus criteria.

Clinical Data

We determined sex and race for all patients. We calculated enrollment APACHE III [21], organ dysfunction using the Brussels score [22] and risk factors for ARDS for both at-risk and ARDS patients. We determined the number of days off mechanical ventilation, days out of the ICU, and days without organ dysfunction, through day 28, using standard methodology [23].

Study Endpoints

For the primary candidate gene SNP analysis, the association of interest was between ARDS and at-risk status for each SNP. Exploratory, secondary candidate gene SNP analysis was restricted to the cohort of patients with ARDS, and the outcome of interest was 28-day mortality (the primary mortality outcome of the ARDS Network trials) with additional exploratory analyses of ventilator-free days and organ-failure-free days to post-enrollment day 28.

Gene Candidates

We evaluated five [5] candidate genes within our candidate pathways (Table 2): 11 SNPs within the GM-CSF pathway and 18 SNPs within the RAGE pathway. We used a TagSNP approach to select candidate SNPs. We selected TagSNP according to the following parameters: linkage disequilibrium (LD) blocks defined using a Caucasian LD map and an $r^2 = 0.8$; Caucasian minor allele frequency (MAF) >0.1; range = −1,500 bps from the initiation codon to +1,500 bps from the termination codon; and 1 SNP/LD bin.

Genotyping Methods

We extracted peripheral blood leukocyte DNA from 842 patients: 690 had ARDS, and 152 were classified as at-risk. We genotyped all markers using a multiplexed bead array assay run on a BeadExpress platform (Illumina, San Diego, California) as part of a larger study. We attained a genotyping call rate of 99.8%. We included 17 blinded internal replicates representing 2.0% of the sample set. The duplicate concordance rate was 99.6%. Because Caucasian patients represented the large majority of patients both locally and within ARDS Network data and there were very few non-Caucasian patients in the *at risk* cohort, we analyzed only samples from Caucasian patients to optimize the signal-to-noise ratio and avoid severe confounding by race.

Statistical Methods

Our primary analysis tested the association between established ARDS vs. at risk status and candidate SNPs (evaluating additive, dominant, and recessive models of inheritance) in a multivariate regression model controlling for clinical covariates. We used multivariable logistic regression models to estimate odds ratios and 95% confidence intervals for associations between genotypes (coded as homozygous wild, heterozygous, homozygous mutant) and ARDS status. We managed the risk of Type 1 statistical error for the primary outcome using a false discovery rate ≤5% that employed the technique of Benjamini and Hochberg [24, 25] adjusted for the number of SNPs evaluated. In exploratory secondary analyses to detect gene:gene interactions, we employed Multivariate Dimension Reduction (MDR) of up to 3 SNPs, with 5-fold cross validation in an additive model of inheritance [26] and Multivariate Adaptive Regression Splines (MARS)[27], which performs objective feature selection, including interaction terms, a technique that addresses usual feature selection problems in simple multivariate logistic regression. We performed statistical analysis and hypothesis testing with the R Statistical Package 3.0.2 (Vienna, Austria).[28]

We estimated power using Quanto 1.2.4.[29] Assuming a baseline ARDS risk of 6.8%,[30] and a ratio of ARDS:at-risk of 4.54, a sample size of 673 cases would have 80% power (with a two-tailed alpha of 0.05) to detect an effect with an Odds Ratio ≥1.6 for a SNP with a Minor Allele Frequency (MAF)≥ 0.16.

Ethical Considerations

This study was approved by the Institutional Review Boards (IRBs) of Intermountain Healthcare, the University of Utah Health Sciences Center, and participating NIH/NHLBI ARDS Network site IRBs. All patients, or their legal surrogates, provided written informed consent.

Results

We identified 842 patients, 152 at-risk individuals and 690 individuals with ARDS, through a process depicted in Figure 1. We obtained non-Utah ARDS Network DNA samples from 548 patients, while 294 samples came from Utah patients. Characteristics of the different populations (at-risk, ARDS cases from Utah centers, ARDS cases from the ARDS Network) are depicted in Table 1. Patients with ARDS were significantly more ill compared to the at-risk group, based on a number of physiologic measures, including APACHE III at enrollment, duration of ICU care and extent of organ failure. Those at-risk for ARDS were more likely to have sepsis and less likely to have pneumonia or aspiration as their identifying risk factor. Mortality among all ARDS patients was 17% (Table 1).

For our primary analysis, we controlled for age (OR 0.98 [0.97–0.995] for each additional year, p 0.007), APACHE III score (OR 1.04 [1.03–1.05] for each additional point, $p <$ 0.001), and presence of sepsis as the risk factor for ARDS (OR 0.13 [0.08–0.20], $p < 0.001$) in multivariate regression. We did not control for PaO_2/FiO_2 ratio at enrollment due to extensive missing data for this parameter. A heatmap displaying correlations among the candidate SNPs is displayed in the eFigure of the online data supplement. No candidate

SNPs were significantly associated with the primary outcome. Our exploratory MDR and MARS analyses did not detect any significant interactions among candidate SNPs.

For our secondary, exploratory analyses, sepsis as risk factor was not associated with mortality and was excluded from the base regression model. After controlling for age (OR 1.03 [1.02–1.05], $p < 0.001$) and APACHE III score (OR 1.04 [1.03–1.04], $p < 0.001$), there was no significant association of candidate SNPs with mortality. One SNP, rs743564 in CSF2, approached significance on a recessive model of inheritance (OR 2.17 [1.25–3.74], unadjusted $p = 0.005$, adjusted p The = 0.15). minor allele frequency (MAF) of rs743564 is 0.224 in a general population of 1000 genomes.[31] In the ARDS cohort, the MAF was 0.39 (0.39 among survivors, 0.43 among non-survivors).

In further exploratory analysis using linear regression of ventilator, ICU-, and organ-free days, there was no significant association with any candidate SNPs, after controlling for age and APACHE III score.

DISCUSSION

In this multicenter cohort of patients with or at risk for ARDS, no candidate SNP within two novel inflammatory or epithelial signaling pathways achieved the pre-specified threshold for significance; only one SNP within CSF2 approached significance on a secondary analysis. While the GM-CSF and RAGE pathways have been associated with important observations about the nature of inflammation and its interaction with epithelial cells, our study did not identify a significant role for genetic variation among the tested SNPs in the development of or outcome after ARDS.

The Receptor for Advanced Glycation Endproducts (RAGE) is a multi-ligand pattern-recognition receptor implicated in inflammation. In mammals, RAGE is predominantly expressed by alveolar epithelial cell [32–34] and transduces the biological impact of discrete families of endogenous danger signals present at sites of cellular injury or death, including advanced glycation end products (AGE), members of the S100/calgranulin family, high mobility group box-1 (HMGB1), membrane-activated complex-1 and amyloid-peptides. By activating RAGE, these ligands up-regulate a program of inflammatory and tissue injury-provoking genes. Although the lung has the capacity to heal fully following ARDS, in many patients there is aberrant repair leading to ongoing inflammation and ultimately to fibrosis. RAGE may promote this fibrotic response through activation of a distinct NADPH oxidase in the lung parenchyma, NOX-4, resulting in production of reactive oxygen species that propagate ongoing inflammation, further injury, and ultimately, fibrosis [12, 34–39].

GM-CSF is a potent growth factor that was originally purified from lung. GM-CSF is a mitogen and survival factor for alveolar epithelial cells [40], and is essential for normal maturation of alveolar macrophages. It is the product of a number of different lung cell types, including alveolar epithelial cells [41, 42]. Lack of GM-CSF leads to pathological accumulation of surfactant and increased susceptibility to pulmonary infection [43–49], while over-expression of GM-CSF has been protective against hyperoxia-induced [10, 50] or bleomycin-induced [51] lung injury in mouse models. There is also some early evidence

that detection of GM-CSF in BAL fluid is associated with improved clinical outcome in human ARDS [52]. One small, early study suggested a trend toward reduced mortality among ARDS patients treated with exogenous GM-CSF [53].

Several gene polymorphisms are associated with greater incidence of ARDS and/or outcome from ARDS, including myosin light chain kinase [54], surfactant protein B [18], mannose binding lectin-2 [17], FAAH [55], and POPDC3 [55].

Strengths of this study include its size, the use of data from multiple centers, and uniform inclusion and exclusion criteria applied to study patients and focus on a series of genes within two discrete pathways.

Limitations of this study include the fact that we did not exhaustively evaluate all polymorphisms in the candidate genes, that we investigated promising targets rather than all possible target genes within the pathways, the possibility that at-risk patients may differ systematically from ARDS patients in terms of non-genetic exposures that were not recorded in the study's case report forms, restriction of at-risk patients to the Utah sites, and lack of racial diversity. We controlled for measured non-genetic differences between the cohorts using multivariate logistic regression. We also acknowledge that the study was not powered to discover variants with modest effect sizes or in variants with low minor allele frequencies. Similarly, ARDS is a syndrome with heterogeneous causes and likely with heterogeneity with respect to mechanistic pathways. We cannot exclude the possibility that gene polymorphisms for the pathways of interest might have effects that may only be identified based on analysis of specific subsets of patients. Our strategy of restricting to 1,500 bps on either side of target genes may have led us to miss important SNPs in the 5′ untranslated region [56, 57] or effects mediated by the 3′ untranslated region, as enhancers may be present as much as 1 million bps from the transcription start site [58]. Our definition of at-risk status was unable to incorporate a granular measure of ARDS risk such as the Lung Injury Prevention Score, published after our present study was underway [30]. Larger studies with more complete sampling of gene candidates within the RAGE and/or GM-CSF pathways, perhaps with a more granular definition of risk, would be required to definitively rule out contributions to the pathophysiology of ARDS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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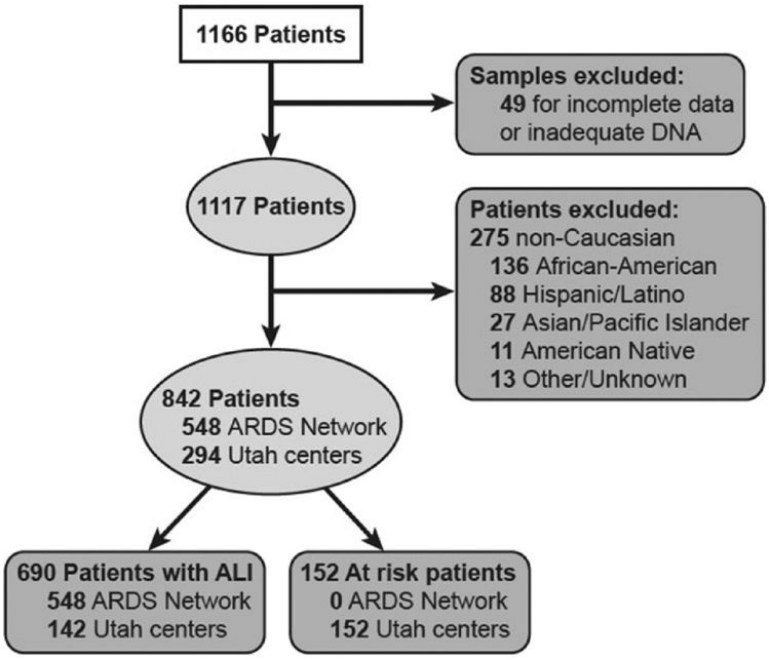

FIGURE 1.
Process by which study patients were defined.

TABLE 1

Demographics and Outcomes Among Three Patient Groups

Variable	At Risk	ARDS Utah	ARDS ARDSNet	p value*
APACHE III at enrollment (points)	70.56	83.14	92.28	<0.001
PF Ratio at enrollment	207.1	130.71	135.28	<0.001
Age (years)	52.85	52.39	50.88	<0.001
Female sex (percent)	51%	56%	47%	0.59
28-day mortality (percent)	4%	14%	18%	<0.001
Risk factor				
Trauma	14%	6%	8%	0.03
Sepsis	58%	21%	24%	<0.001
Transfusion	3%	4%	2%	0.38
Aspiration	2%	14%	18%	<0.001
Pneumonia	19%	49%	41%	<0.001
Ventilator-free days	25.57	17.44	15.21	<0.001
ICU-free days	17.5	11.92	14.21	<0.001
Organ-failure free days	27.29	26.25	10.93	<0.001

ARDS = Acute Respiratory Distress Syndrome; ARDSNet = National Institutes of Health ARDS Clinical Trials Network.

*For comparison of all ARDS vs. all at risk patients.

TABLE 2

Candidate Genes and SNPs

Pathway	Gene	SNP	MAF*	Genotype	ARDS (N)	At risk (N)	OR	p value**
GM-CSF	CSF2	rs2069616	0.43	AA	241	48	1	NA
				AG	316	69	0.99	0.66
				GG	130	35	0.96	0.22
		rs25881	0.17	GG	473	101	1	NA
				AG	190	43	0.99	0.77
				AA	23	5	1	0.97
		rs25882	0.2	AA	437	97	1	NA
				AG	208	45	1	0.9
				GG	32	8	0.98	0.77
		rs27438	0.21	GG	436	94	1	NA
				AG	219	50	0.99	0.77
				AA	33	8	0.98	0.78
		rs743564	0.4	AA	262	55	1	NA
				AG	311	66	1	0.96
				GG	117	31	0.96	0.35
	CSF2RB	rs10222232	0.48	GG	188	43	1	NA
				AG	338	75	1.00	0.89
				AA	158	32	1.02	0.64
		rs11705394	0.25	AA	368	82	1	NA
				AG	263	59	1	0.97
				GG	30	9	0.95	0.45
		rs131842	0.17	AA	468	104	1	NA
				AG	205	42	1.01	0.69
				GG	17	6	0.92	0.33
		rs1534880	0.44	GG	226	40	1	NA
				CG	335	82	0.95	0.13
				CC	129	30	0.96	0.32
		rs738149	0.29	GG	336	86	1	NA
				AG	292	52	1.05	0.06
				AA	59	13	1.02	0.64
		rs909486	0.45	GG	212	48	1	NA
				AG	335	70	1.01	0.7
				AA	142	34	0.99	0.82
RAGE	AGER	rs3131300	0.17	AA	475	103	1	NA
				AG	192	41	1	0.94
				GG	17	8	0.87	0.07
		rs3134943	0.15	GG	503	107	1	NA
				AG	165	42	0.97	0.37
				AA	22	3	1.06	0.48

Pathway	Gene	SNP	MAF*	Genotype	ARDS (N)	At risk (N)	OR	p value**
	HMGB1	rs3742305	0.23	GG	409	86	1	NA
				CG	241	56	0.99	0.6
				CC	39	8	1	0.95
	NOX4	rs10830277	0.12	AA	528	126	1	NA
				AG	148	23	1.06	0.08
				GG	14	3	1.02	0.86
		rs11821838	0.05	GG	619	142	1	NA
				AG	67	9	1.07	0.14
				AA	3	1	0.94	0.74
		rs12799930	0.35	AA	277	69	1	NA
				AC	319	66	1.03	0.33
				CC	81	14	1.05	0.24
		rs1827428	0.08	GG	567	116	1	NA
				AG	96	20	1	0.95
				AA	4	1	0.97	0.86
		rs1847137	0.32	CC	319	77	1	NA
				CG	291	57	1.03	0.28
				GG	75	15	1.03	0.54
		rs2164521	0.12	GG	535	121	1	NA
				AG	141	31	1	0.9
				AA	13	0	1.2	0.09
		rs2202150	0.39	AA	245	65	1	NA
				AG	338	66	1.05	0.11
				GG	107	21	1.05	0.26
		rs317150	0.09	GG	560	131	1	NA
				AG	120	20	1.05	0.19
				AA	7	1	1.07	0.64
		rs317155	0.45	AA	204	53	1	NA
				AG	329	74	1.02	0.46
				GG	156	25	1.07	0.07
		rs317187	0.4	AA	247	63	1	NA
				AC	322	70	1.02	0.4
				CC	120	19	1.07	0.09
		rs319029	0.41	GG	209	36	1	NA
				CG	222	45	0.98	0.5
				CC	107	19	1	0.92
		rs319030	0.45	GG	198	53	1	NA
				AG	340	76	1.03	0.35
				AA	150	23	1.08	0.04
		rs521765	0.15	GG	492	114	1	NA
				CG	178	37	1.02	0.6
				CC	19	1	1.15	0.11

Pathway	Gene	SNP	MAF*	Genotype	ARDS (N)	At risk (N)	OR	p value**
		rs595518	0.17	CC	463	106	1	NA
				AC	197	43	1.01	0.81
				AA	18	2	1.09	0.33
		rs957140	0.46	GG	189	51	1	NA
				AG	361	73	1.05	0.15
				AA	138	28	1.04	0.26

SNP = Single Nucleotide Polymorphism; MAF = Minor Allele Frequency; ARDS = Acute Respiratory Distress Syndrome; OR = Odd; Ratio

* MAF determined from the study sample, both cases and controls.

** P value and Odds Ratio determined from univariate logistic regression.