The Association Between a *Darc* Gene Polymorphism and Clinical Outcomes in African American Patients With Acute Lung Injury

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Background: Acute lung injury (ALI) mortality is increased among African Americans compared with Americans of European descent, and genetic factors may be involved. A functional *T-46C* polymorphism (rs2814778) in the promoter region of Duffy antigen/receptor for chemokines (*Darc*) gene, present almost exclusively in people of African descent, results in isolated erythrocyte DARC deficiency and has been implicated in ALI pathogenesis in preclinical and murine models, possibly because of an increase in circulating Duffy-binding, proinflammatory chemokines like IL-8. We sought to determine the effect of the functional rs2814778 polymorphism, *C/C* genotype (Duffy null state), on clinical outcomes in African Americans with acute lung injury.

Methods: Clinical data and biologic specimens from African American patients with ALI who enrolled in three randomized controlled trials were analyzed. Multivariate analysis accounted for proportion of African ancestry, sex, cirrhosis, and severity of illness on presentation.

Results: Among 132 subjects, 88 (67%) were Duffy null (*C/C* genotype). The Duffy null state was associated with a 17% absolute risk increase (95% CI, 1.4%-33%) in mortality at 60 days, a median of 8 fewer ventilator-free days (95% CI, 1-18.5), and 4.5 fewer organ failure-free days (95% CI, 0-18) compared with individuals with the *C/T* or *T/T* genotypes (all *P* values < .05). Estimates were similar on multivariate analysis. In African Americans without the null variant, clinical outcomes were similar to those in patients of European descent. A subgroup analysis suggested that plasma IL-8 levels are increased in Duffy null individuals.

Conclusions: Our results provide evidence that the functional rs2814778 polymorphism in the gene encoding DARC is associated with worse clinical outcomes among African Americans with ALI, possibly via an increase in circulating IL-8.

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Abbreviations: AIM = ancestry informative marker; ALI = acute lung injury; APACHE = Acute Physiology and Chronic Health Evaluation; APS = Ancestry Proportion Score; DARC = Duffy antigen/receptor for chemokines; IQR = interquartile range; OFF = organ failure free; SNP = single-nucleotide polymorphism; VFD = ventilator-free day

Acute lung injury (ALI) is a common and frequently fatal cause of acute respiratory failure, affecting up to 20% of patients who are mechanically ventilated and carrying mortality rates from 30% to 40%.¹⁻³ The burden of ALI morbidity and mortality affects African Americans disproportionately: Compared with Americans of European descent, African Americans suffer at least 50% increased risk of death.^{4,5} Although it is not known to what extent genetic sus-

ceptibility contributes to differences in ALI mortality, African descent has been associated with a “high-expression profile” of proinflammatory chemokines

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and a “low-expression profile” of antiinflammatory chemokines.^{6,7} Further, current evidence indicates that the proximal cause of ALI is excessive production of

proinflammatory chemokines with neutrophil influx in the lungs.⁸

The role of Duffy antigen/receptor for chemokines (DARC) is of particular interest because a *T-46C* polymorphism⁹ in the promoter region of the *Darc* gene (rs2814778; chr 1: 159174683-159174683; HG build 19),¹⁰ present as the *C/C* genotype almost exclusively among people of African descent, results in selective deficiency of erythrocyte expression of the DARC.^{9,11} Erythrocyte DARC has historically been known as a minor blood group type and as the receptor for *Plasmodium vivax* malaria.¹²⁻¹⁵ The *C/C* genotype, called the “Duffy null” state, is hypothesized to confer a survival advantage that has resulted in selection of this phenotype in West African populations.⁹ The Duffy null state is present in approximately two-thirds of African Americans due to racial admixture; in contrast, the Duffy null state is exceedingly rare in people of European descent.^{16,17}

Unlike other chemokine receptors, the binding of erythrocyte DARC by target chemokines does not induce intracellular signal transduction.¹⁸ Rather, both murine and preclinical data suggest that erythrocyte DARC modulates chemokine homeostasis by acting as a “chemokine reservoir,”¹⁹⁻²² binding proinflammatory chemokines such as IL-8 when concentration levels are high during tissue inflammation and then releasing them systemically when levels are low.²³⁻²⁵ In support of this proposed role, murine

and in vitro human studies demonstrate that plasma IL-8 levels and alveolar neutrophil migration are significantly increased in DARC-deficient models following lipopolysaccharide stimulation compared with DARC-positive models.^{23,26-28} In clinical studies, the Duffy null state has been associated with baseline neutropenia, altered chemokine levels in endotoxemia, and increased susceptibility to HIV and asthma.^{7,29-33} Given the evidence that erythrocyte DARC deficiency alters host chemokine response and increases susceptibility to inflammatory disease, we sought to determine whether there was an association between the Duffy null state and ALI outcomes.

MATERIALS AND METHODS

Subjects

This cohort study analyzed data from African Americans enrolled in three multicenter, randomized, controlled trials conducted by the ARDS Network evaluating therapeutic interventions for ALI. The three trials included in this study are shown in Table 1; details have been previously published.³⁴⁻³⁶ Briefly, eligible patients met diagnostic criteria for ALI and required mechanical ventilation, and the three trials used similar inclusion and exclusion criteria.³⁷ All participants or their surrogates were asked to co-enroll in an ancillary study designed to study the role of genetic markers. The institutional review boards of each participating hospital reviewed and approved the primary and ancillary studies (see cited articles for details on IRB approval process).³⁴⁻³⁶ Written informed consent was obtained from participants or legally authorized surrogates. DNA was extracted from whole blood and made available for this study by the ARDS Network DNA repository in the Center for Human Genetics Research at Vanderbilt University. The DNA samples were linked to the phenotypic data by an encrypted key provided by the ARDS network, and no patient identifiers were provided. Therefore, this analysis was deemed as not human research. We limited our study to patients self- or surrogate-identified African American, because the Duffy null genotype is too rare in other populations to draw meaningful conclusions. Of a total of 340 African American patients enrolled in the three trials (Fig 1), we excluded 208 participants for whom DNA was not available. In total, this study had 132 participants.

Genotyping

The *Darc* polymorphism (rs2814778) was genotyped using the TaqMan-based allelic discrimination method³⁸ using single-nucleotide polymorphism (SNP) genotyping assay supplied by Applied Biosystems (primer and probe sequences are available on request [saprua@peds.ucsf.edu]). Patients with two copies of the *C* allele were labeled Duffy null, whereas patients with one or zero copies were labeled Duffy positive, in convention with well-described erythrocyte DARC expression.⁹ Seventy-two ancestry informative markers (AIMs) were genotyped using a template-directed dye-terminator incorporation method on the Beckman Coulter SNPstream 48-plex platform.³⁹

Outcomes

Outcomes included (1) 60-day mortality, wherein patients discharged home without the use of assisted ventilation before day 60 were assumed alive at 60 days,³⁵ and (2) days of failure of the pulmonary and nonpulmonary organ systems through

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*A complete list of study participants is located in e-Appendix 1.

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Table 1—Characteristics of ARDS Network Randomized Controlled Trials Included in This Analysis

Trial	ALVEOLI	FACTT	ALTA
Years	1999-2002	2000-2005	2007-2008
Intervention	Higher vs lower PEEP	Pulmonary artery catheter vs central venous catheter and conservative vs liberal fluid management strategy	Aerosolized albuterol vs placebo
Patients enrolled, No.	549	1,000	282
African American patients enrolled, No.	77	217	46
Did intervention(s) reduce mortality?	No	No ^a	No

ALTA = Albuterol for the Treatment of Acute Lung Injury; ALVEOLI = Assessment of Low Tidal Volume and Elevated End-Expiratory Volume to Obviate Lung Injury; FACTT = Fluid and Catheter Treatment Trial; PEEP = positive end-expiratory pressure; VFD = ventilator-free day.

^aFluid conservative approach was associated with a higher number of VFDs, but survival was similar.

day 28 or discharge. Pulmonary organ failure was measured by ventilator-free days (VFDs), defined as number of days free of mechanical ventilation to day 28, with VFD = 0 for patients who died in the first 4 weeks.⁴⁰ Nonpulmonary organ failure-free (OFF) days were calculated by subtracting the number of days patients met any Brussels organ failure criteria⁴¹ from the lesser of 28 days or number of days to death.³ A lower number of VFDs and OFF days represents a worse outcome, and organs and systems were considered failure-free after patients were discharged from the hospital.

Ancestry Proportion Score

Because patients with increased African Ancestry are more likely to have the null C/C genotype,⁴² we considered the possibility of confounding by genetic ancestry, whereby any association between rs2814778 and clinical outcomes could be caused by another factor related to proportion of African descent.⁴³ We used a standard method using AIMs to calculate the proportion of African ancestry (Ancestry Proportion Score [APS]) for each patient, which was included as a covariate in our multivariate analysis (details in e-Appendix 2).⁴⁴⁻⁴⁶

Statistical Analysis

Demographic and baseline clinical characteristics according to Duffy status were compared using a χ^2 for dichotomous variables and *t* test or Wilcoxon rank-sum test for continuous variables. Hardy-Weinberg equilibrium was calculated overall and in survivors.

To assess differences in clinical outcomes between Duffy-null and Duffy-positive patients, we first examined unadjusted differences using χ^2 and the Wilcoxon rank-sum tests. Δ -Method standard error was used to obtain the normal theory CIs for the mortality risk difference. The bootstrapping method was used to provide a bias-corrected percentile CI for differences in medians for VFDs and OFF days. A Kaplan-Meier survival curve was generated for mortality. In adjusted analyses, we used logistic regression to generate OR for 60-day mortality and zero-inflated Poisson regression with robust SEs to generate rate ratios for VFDs and OFF days. In addition to APS, covariates associated with Duffy status with *P* values < .3 were considered for multivariate analysis. We adjusted for APS, sex, and cirrhosis to account for likely confounders. In a separate model, we adjusted for APACHE (Acute Physiology and Chronic Health Evaluation) III⁴⁷ to determine the association between Duffy status and hospital outcomes independent of initial severity of illness. Because we hypothesized that Duffy status is likely to affect both severity of illness and clinical outcomes through a similar pathway, we did not adjust for APACHE III in the primary model.

In a secondary analysis, we examined whether the effects of Duffy status differed among patients with an infectious cause of ALI, such as sepsis and pneumonia. We also compared ALI out-

comes among Duffy-positive African Americans in our study to European Americans, presumed to be Duffy positive, enrolled in the same ARDS Network trials. Finally, in a subgroup analysis of patients enrolled in two trials with measured plasma levels of IL-8,^{34,36} we tested the association (1) between Duffy status on IL-8, and (2) plasma IL-8 levels on clinical outcomes. The Wilcoxon rank-sum test was used for these analyses.

The analyses were performed using STATA version 10 (STATA Corp). Statistical significance was defined as a two-tailed *P* < .05 for all analyses (further detail included in e-Appendix 2).

RESULTS

Baseline Patient Characteristics

Of the 132 African American patients included in this study, 88 (67%) were Duffy null (C/C genotype), a finding consistent with reported prevalence among African Americans.⁴⁸ Table 2 shows that the Duffy-null and Duffy-positive groups were comparable in demographics and clinical characteristics, including age, sex, and study of enrollment, as well as baseline

FIGURE 1. Flowchart of inclusion and exclusion criteria. * = ARDS Network Trials: ALVEOLI (Assessment of Low Tidal Volume and Increased End-Expiratory Volume to Obviate Lung Injury), FACTT (Fluid and Catheter Treatment Trial), and ALTA (Albuterol for the Treatment of Acute Lung Injury) trials. NHLBI = National Heart, Lung, and Blood Institute.

Table 2—Baseline Characteristics African American Patients With ALI (N = 132)

Characteristic	Duffy Null (n = 88)	Duffy Positive (n = 44)	P Value
Demographics			
Age, y	50.8 ± 16.3	49.7 ± 15.3	.71
Male sex	50 (57)	20 (45)	.22
ARDS Network Study			.96
ALVEOLI	2 (2)	1 (2)	
FACTT	62 (70)	32 (73)	
ALTA	24 (27)	11 (25)	
FACTT fluid conservative arm ^a	27 (44)	15 (47)	.76
Clinical characteristics			
Comorbidities			
HIV/AIDs	9 (10)	6 (14)	.53
Cancer	3 (3)	1 (2)	.72
Diabetes	26 (30)	10 (23)	.45
Cirrhosis	8 (9)	1 (2)	.14
End-stage renal disease	1 (1)	1 (2)	.60
Primary cause of lung injury			.57
Trauma	3 (3)	0	.22
Sepsis	21 (24)	8 (18)	.46
Transfusion	1 (1)	1 (2)	.61
Aspiration	15 (17)	6 (14)	.61
Pneumonia	43 (49)	24 (55)	.54
Other	5 (6)	5 (11)	.24
Severity of illness			
WBC count			
24-h High WBC, median (IQR), 10 ⁶ /mL	12.4 (6.9-17.2)	14.7 (6.9-18.3)	.64
24-h Low WBC, median (IQR), 10 ⁶ /mL	9.7 (5.8-15.2)	12.4 (4.3-15.2)	.60
Vasopressor use	33 (38)	16 (36)	.90
PaO ₂ /Fio ₂ , median (IQR)	120 (82-183)	112 (87-167)	.52
Oxygenation index, median (IQR)	13.0 (7.1-22.0)	11.1 (7.9-18.8)	.81
APACHE III score	106 ± 32	98 ± 28	.19
Lung injury score ^b	2.8 ± 0.7	2.8 ± 0.7	.97

Values are means ± SD or No. (%) unless otherwise noted. ALI = acute lung injury; APACHE = Acute Physiology and Chronic Health Evaluation; IQR = interquartile range. See Table 1 for expansion of other abbreviations.

^aOut of a total of 94 patients from the FACTT trial.

^bLung injury score is the four-point score described in reference.

comorbidities. Pneumonia, sepsis, and aspiration were the top three causes of ALI in both Duffy groups. There was no difference in median high and low WBC count in the first 24 h of enrollment and no difference in the proportion of patients with a WBC count that met systemic inflammatory response syndrome criteria (< 4 or $> 12 \times 10^6/\text{mL}$; $P = .39$). Other measures of baseline severity, including vasopressor use, PaO₂/Fio₂ ratio, oxygenation index, APACHE III score, and lung injury score were also similar. Compared with the 208 African American patients excluded for lack of DNA, our study patients differed according to some baseline clinical characteristics (e-Tables 1, 2); however, there were no differences in any clinical outcome across groups.

Allelic and Genotypic Frequencies of rs2814778 and Ancestry Proportion Score

Hardy-Weinberg equilibrium was observed among participants who survived ($P = .24$), but the prevalence of the Duffy null genotype (C/C) was greater

among those who died compared with those who survived (Table 3), resulting in observed overall Hardy-Weinberg disequilibrium ($P = .03$).

The proportion of African ancestry varied widely in this sample of African Americans, with an average proportion of African ancestry of 0.82 ± 0.15 (median, 0.85; interquartile range [IQR], 0.76-0.91) (Fig 2A). The C allele was significantly associated with increased estimated African ancestry (Fig 2B) ($P = .008$); however, the APS was similar among patients who died (median,

Table 3—Gene Encoding DARC rs2814778 Genotype and Allele Frequencies Among 132 African American Patients With ALI

Patients	Genotype Frequency			C Allele Frequency
	C/C	C/T	T/T	
Overall	0.67	0.26	0.07	0.80
Nonsurvivors	0.79	0.14	0.07	0.86
Survivors	0.61	0.31	0.08	0.77

DARC = Duffy antigen/receptor for chemokines. See Table 2 legend for expansion of other abbreviation.

FIGURE 2. A, Estimated ancestry proportion score (APS) in ancestral African (HapMap CEPH-YRI), ancestral European (HapMap CEPH-CEU), and in African American ARDS Network study sample; each black line represents estimated APS for each individual. Although ancestral populations derived on HapMap exhibited relatively uniform proportions of African ancestry, the proportion of African ancestry varied widely in this sample of African Americans. Mean APS: Ancestral African = 0.99; Ancestral European = 0.02; ARDS Network sample = 0.82. B, Comparison of proportion African ancestry among African Americans in ARDS Network according to *Darc* rs2814778 genotype. The line in the middle of the box represents the median and the lines that form the box correspond to the 25th and 75th percentiles. *Kruskal-Wallis equality-of-populations rank test. The C allele was significantly associated with increased estimated African ancestry ($P = .008$), with a median APS of 0.87 among patients with the C/C genotype, 0.82 among those with the C/T genotype, and 0.72 among those with the T/T genotype.

0.86; IQR, 0.78-0.95) and those who lived (median, 0.84; IQR, 0.75-0.90) ($P = .27$).

Duffy Status and Clinical Outcomes

Compared with 21% mortality in Duffy-positive individuals, 38% of the Duffy-null patients died, representing a 17% absolute risk increase of death (95% CI, 1.4%-33%; $P = .04$) (Fig 3, Table 4). In unadjusted analysis, the Duffy-null state was associated with 2.6-fold increased odds of death at 60 days (95% CI, 1.1-6.3; $P = .03$) compared with Duffy-positive individuals. This association did not substantively change after adjustment for ancestry, sex, and cirrhosis or with additional adjustment for APACHE III (Table 5). There was no evidence that the effect of the Duffy polymorphism on mortality differed by infectious vs noninfectious cause ($P = .44$ for heterogeneity).

Duffy-null patients had a median of 8 fewer VFDs (95% CI, 1.0-18.5) compared with those who were Duffy positive (Table 4). Using zero-inflated Poisson regression, the Duffy-null state was associated with a 24% reduction in VFDs (95% CI, 13%-33%; $P = .0001$) in unadjusted analysis (Table 5). Adjustment for

ancestry, sex, and cirrhosis slightly moderated this effect, with a 19% reduction in VFDs (95% CI, 7%-29%; $P = .002$) observed in Duffy-null individuals in comparison with those with a Duffy-positive genotype. The estimate did not change after further adjustment for APACHE III (Table 5). Among just

FIGURE 3. Probability of survival to hospital discharge during the first 60 days according to Duffy status. Duffy-null individuals have increased risk of in-hospital death across the follow-up period. This is statistically significant ($P = .04$).

Table 4—Duffy Status and Clinical Outcomes in African American Patients in ARDS Network

	Duffy Null (n = 88)	Duffy Positive (n = 44)	P Value
Death by day 60, No. (%)	33 (38)	9 (21)	.04
VFDs	13 (0-23)	21 (0-26)	.04
VFDs among survivors ^a	20 (11-24)	24 (17-26)	.07
Organ FFDs	0 (0-13.5)	4.5 (0-21)	.04
CNS FFDs	6.5 (0-22)	21.5 (4.5-23)	.01
Cardiovascular FFDs	22 (8.5-25.5)	26 (17.5-27)	.01
Hepatic FFDs	28 (9.5-28)	28 (20.5-28)	.06
Coagulation FFDs	24.5 (9-28)	28 (19.5-28)	.10
Renal FFDs	19.5 (5.5-28)	26.5 (11.5-28)	.19

Values are median (IQR) unless otherwise noted. FFD = failure-free day. See Table 1 legend for expansion of other abbreviation.

^aAmong survivors, Duffy null = 55, Duffy positive = 35.

the 90 survivors, reduction in VFD remained statistically significant in all analyses (Table 5).

Duffy-null individuals also had a median of 4.5 fewer OFF days (95% CI, 0.0-18) compared with Duffy-positive individuals. The Duffy-null state was associated with a 23% reduction (95% CI, 1% to 41%; $P = .04$) in OFF days. This estimate was not substantively changed in multivariate analysis (Table 5). There was a trend toward decreased organ-specific failure-free days among Duffy-null patients in all organ systems (Tables 4, 5). However, we observed the strongest association in the central nervous and cardiovascular systems.

Comparison of Duffy-Positive African and European Americans

As reported previously,⁴ African Americans in the ARDS Network trials had increased mortality,

increased mechanical ventilation requirements, and increased organ failure (data not shown). We hypothesized that poor clinical outcomes in African Americans may have been driven by Duffy-null individuals and that Duffy-positive African Americans would have similar clinical outcomes to European Americans. In comparisons of Duffy-positive African (N = 44) and European (N = 1,278) Americans enrolled in the three ARDS Network trials, there were no differences in mortality, VFD, or OFF days (Table 6), and Duffy-positive African Americans had a trend toward improved outcomes across outcomes assessed.

Subgroup Analysis of Plasma IL-8 Levels According to Duffy Status and Clinical Outcomes

Based on the hypothesis that erythrocyte-bound DARC acts as a chemokine reservoir, the expected effect of erythrocyte DARC deficiency on plasma

Table 5—Multivariate Analysis of Duff-Null State on Clinical Outcomes in African American Patients Compared With Duffy-Positive Referent in ARDS Network

Outcomes	Unadjusted	Adjusted for Ancestry	Adjusted for Ancestry, Sex, Cirrhosis	Adjusted for Ancestry, Sex, Cirrhosis, and APACHE III
Death by day 60, OR (95% CI)	2.62 (1.09-6.34) $P = .03$	2.70 (1.06-6.88) $P = .04$	2.67 (1.02-6.95) $P = .04$	2.65 (0.97-7.27) $P = .06$
VFDs, rate ratio (95% CI)	0.76 (0.67-0.87) $P = .0001$	0.78 (0.68-0.89) $P = .0002$	0.81 (0.71-0.93) $P = .002$	0.83 (0.73-0.94) $P = .004$
VFDs among survivors ^a	0.84 (0.74-0.95) $P = .005$	0.84 (0.74-0.96) $P = .008$	0.86 (0.76-0.98) $P = .02$	0.87 (0.77-0.98) $P = .03$
Organ FFDs, rate ratio (95% CI)	0.77 (0.59-0.99) $P = 0.04$	0.76 (0.57-1.00) $P = .05$	0.77 (0.58-1.01) $P = .07$	0.74 (0.58-0.96) $P = .02$
CNS FFDs	0.79 (0.65-0.96) $P = .02$	0.78 (0.63-0.98) $P = .03$	0.82 (0.65-1.02) $P = .007$	0.83 (0.67-1.03) $P = .09$
Cardiovascular FFDs	0.87 (0.76-1.00) $P = .06$	0.90 (0.77-1.04) $P = .17$	0.91 (0.78-1.06) $P = .022$	0.92 (0.80-1.06) $P = .25$
Hepatic FFDs	0.91 (0.81-1.02) $P = .11$	0.91 (0.80-1.03) $P = .14$	0.91 (0.80-1.04) $P = .17$	0.92 (0.82-1.04) $P = .20$
Coagulation FFDs	0.91 (0.79-1.04) $P = .16$	0.93 (0.81-1.08) $P = .35$	0.95 (0.83-1.10) $P = .53$	0.96 (0.84-1.10) $P = .56$
Renal FFDs	0.94 (0.81-1.10) $P = .46$	0.93 (0.79-1.10) $P = .39$	0.94 (0.79-1.11) $P = .45$	0.95 (0.82-1.10) $P = .50$

See Table 1, 2, and 4 legends for expansion of abbreviations.

^aAmong survivors, Duffy null = 55, Duffy positive = 35.

Table 6—Clinical Outcomes Among European American Patients Compared With Duffy-Positive Patients in the Study Sample

Outcomes	European American (n = 1278)	Duffy-Positive African American (n = 44)	P Value
Death by day 60, No. (%)	299 (23)	9 (21)	.65
VFDs	19 (1-24)	21 (0-26)	.29
VFDs among survivors ^a	21 (16-25)	24 (17-26)	.12
Organ FFDs	1 (0-17)	4.5 (0-21)	.25
CNS failure	18 (1-25)	21.5 (4.5-23)	.64
Cardiovascular FFDs	24 (15-27)	26 (17.5-27)	.21
Hepatic FFDs	28 (19-28)	28 (20.5-28)	.53
Coagulation FFDs	28 (21-28)	28 (19.5-28)	.89
Renal FFDs	28 (18-28)	26.5 (11.5-28)	.11

Data given as median (IQR) unless otherwise indicated. See Table 1 and 4 legends for expansion of abbreviations.

^aAmong survivors, total European American = 978, Duffy-positive African American = 35

IL-8 levels would be an increase in circulating levels as a result of reduced IL-8 binding. Among 37 patients with measured plasma IL-8 levels, we found a strong trend toward increased IL-8 at day 0 ($P = .07$) and a statistically significant increase in IL-8 at follow-up day 3 ($P = .04$) (Table 7) among Duffy-null compared with Duffy-positive individuals. In addition, IL-8 levels were strongly associated with death (day 0, $P = .001$; day 3, $P = .003$) (Table 8), decreased VFDs (day 0, $P < .0001$; day 3, $P < .0001$), and decreased OFF days (day 0, $P = .03$; day 3, $P = .0007$).

DISCUSSION

In this study of African Americans with ALI enrolled in three randomized controlled trials, patients with the Duffy-null state (C/C genotype, rs2814778) were at increased risk for mortality at 60 days, required increased days of mechanical ventilation, and had increased organ failure in comparison with their Duffy-positive counterparts. These findings were not confounded by proportion of African ancestry, sex, presence of cirrhosis, or severity of illness at baseline. Further, we found that clinical outcomes were similar among Duffy-positive African Americans compared with European Americans. Moreover, in a small subgroup analysis, we found a strong trend toward increased baseline IL-8 among patients with

the Duffy-null genotype. On follow-up day 3, this association was statistically significant. Finally, in contrast to differences in WBC count according to Duffy status that are well described in non-critically ill patients,^{7,30,33} we did not find a difference in WBC count in this ALI cohort.

The results of this study provide evidence of the association between the Duffy-null state and clinical outcomes in ALI. Although the Duffy-null state is already known to cause baseline neutropenia and has been implicated in asthma and HIV susceptibility in individuals of African descent, this study builds on both murine and human preclinical research showing that erythrocyte-bound DARC is involved in chemokine homeostasis and alveolar neutrophil migration in endotoxemia models. In the context of these data, there is strong biologic plausibility that erythrocyte-bound DARC plays an important role in the host immune response involved in the pathogenesis of ALI. Further, our study provides corroborating biologic support for the hypothesis that the effect of the Duffy-null polymorphism on clinical outcomes in ALI may be mediated through increases in circulating IL-8 in response to inflammatory stimuli. The absence of DARC on erythrocytes may lead to increased levels of DARC-binding inflammatory chemokines migrating to the site of injury, resulting in increased endothelial and epithelial injury. Last, the lack of difference in WBC according to Duffy status is of interest given the well-described differences in neutrophil count at baseline.^{7,30,31,49} However, Duffy-associated differences in leukocyte count have not been previously tested in a critical illness population. A similar effect was observed in an endotoxemia study in which baseline differences according to Duffy status were observed, but postendotoxin differences were not.⁷ It is possible that Duffy-null individuals have an increased change from baseline in the neutrophil response, but we do not have pre-illness data to support this in ALI.

Table 7—Day 0 and Day 3 IL-8 Levels in Patients Enrolled in ALVEOLI and ALTA According to Duffy Status

Biomarker	Duffy Null (n = 25)	Duffy Positive (n = 12)	P Value
IL-8, day 0	155 (56-419)	51 (35-90)	.07
IL-8, day 3 ^a	78 (39-132)	34 (25-69)	.04

Data are presented as median (IQR). Wilcoxon rank-sum statistical test. See Table 1 and 2 legends for expansion of abbreviations.

^aDuffy null = 24, Duffy positive = 11.

Table 8—Day 0 and Day 3 IL-8 in 37 ALVEOLI and ALTA Patients According to Death at 60 d

Biomarker	Died (n = 11)	Lived (n = 26)	P Value
IL-8, day 0	345 (154-1,538)	54 (33-99)	.001
IL-8, day 3	132 (114-249)	44 (28-69)	.0003

Data are presented as median (IQR). Wilcoxon rank sum statistical test. See Table 1 and 2 legends for expansion of abbreviations.

Racial disparities in ALI outcomes have been hypothesized to be partly attributable to decreased access to care resulting in delays in presentation and increased severity of illness on presentation.^{4,50} We found an association between Duffy status and clinical outcomes that was, to some extent, independent of the severity of illness at baseline. This finding suggests there may be differences in the course of ALI and response to treatment in Duffy-null patients. On the other hand, we also found some weak evidence that the Duffy-null state may be associated with severity of illness, with a borderline association with increased APACHE III score ($P = .19$). It is plausible that differences in chemokine response affecting clinical outcomes are likely to also affect severity of illness on presentation, but further studies are required to determine if Duffy status plays a role in the increased severity of illness observed in African American patients with ALI.

We limited this study to an African American population. Prior studies comparing African Americans with other racial groups may have been confounded by social, economic, and cultural factors that differ by race. Some residual confounding may arise in our sample from differences in admixture among African Americans, but we adjusted for this potential confounder. The proportion of African ancestry varied widely in our study, which is characteristic of an admixed population, and the mean proportion observed of 82% African ancestry is consistent with prior estimates for African Americans using admixture mapping techniques.³¹

Our study has some limitations. Although this cohort is one of the largest samples of African American patients with ALI, the sample size was modest. However, few potential confounders were associated with Duffy status, and racial or socioeconomic differences in treatment should be minimized in the setting of clinical trials. DNA was available for only 39% of African Americans enrolled in the ARDS Network trials, primarily because DNA collection in the earlier two trials was sporadic. However, clinical outcomes among the patients without DNA were similar to those we included, demonstrating that our study sample is representative of African Americans enrolled in the ARDS Network. Additionally, our comparison of clinical outcomes in Duffy-positive

African Americans to the presumed Duffy-positive European Americans has limited power. Further, although we have evaluated only one SNP within the *Darc* gene, it is well established that variation in rs2814778 accounts for nearly all of the variation in expression of DARC on erythrocytes; therefore, testing of DARC expression was not critical.^{9,51,52} In addition, although basic, preclinical, and our subgroup data support the biologic plausibility of the rs2814778 polymorphism in ALI pathogenesis, we have only limited data from this study cohort on the association between Duffy status and IL-8 from which to draw conclusions on the molecular effects of this polymorphism. Finally, we have not validated our findings in an external cohort. Although we only tested one polymorphism and multiple hypothesis testing is not an important issue in this case, external validation is nonetheless the next logical step in further testing the effect of Duffy status on clinical outcomes in ALI.

In summary, our findings indicate that the common rs2814778, *Darc* C/C genotype, present in the majority of African Americans, may play a role in the pathogenesis of ALI and may partially account for the increased mortality among African American patients. These findings may have important clinical implications both for risk prediction, since the Duffy null phenotype—a minor blood group type—can be readily tested in real time,^{9,51,52} and for development of targeted treatments. Future work should include testing of this association in an independent ALI cohort and determination of the molecular effects of Duffy status on chemokine homeostasis in clinical studies.

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Dr Kangelaris: contributed to study design, data analysis and interpretation, drafting and revising the manuscript critically for important intellectual content, and approving the version to be published.

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