Published in final edited form as:

Transl Res. 2017 February ; 180: 12–21. doi:10.1016/j.trsl.2016.07.020.

Epigenetic Contribution of the Myosin Light Chain Kinase Gene to the Risk for Acute Respiratory Distress Syndrome

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Abstract

Acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome with a considerable case fatality rate (~30-40%). Health disparities exist with African descent subjects (ADs) exhibiting greater mortality than European descent individuals (EDs). Myosin light chain kinase (MLCK) is encoded by *MYLK* whose genetic variants are implicated in ARDS pathogenesis and may influence ARDS mortality. As baseline population-specific epigenetic changes, i.e. cytosine modifications, have been observed between AD and ED individuals, epigenetic variations in *MYLK* may provide insights into ARDS disparities. We compared methylation levels of *MYLK* CpGs between ARDS patients and ICU controls overall and by ethnicity in a nested case control study of 39 ARDS cases and 75 non-ARDS intensive care unit controls. Two *MYLK* CpG sites (cg03892735, cg23344121) were differentially modified between

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All authors have read the journal's policy on disclosure of potential conflicts of interest and have no potential conflicts of interest to declare. There were no sources of editorial support for the preparation of this manuscript. All authors have read the journal's authorship agreement and the manuscript has been reviewed and approved by all named authors.

ARDS subjects and controls ($p<0.05$; $q<0.25$) in a logistic regression model, where no effect modification from ethnicity or age was found. One CpG site was associated with ARDS in patients less than 58 years old, cg19611163 (intron 19,20). Two CpG sites were associated with ARDS in EDs only, gene body CpG (cg01894985, intron 2,3) and CpG (cg16212219, intron 31,32), with higher modification levels exhibited in ARDS subjects than controls. *Cis*-acting mQTL (modified cytosine quantitative trait loci) were identified using linear regression between local genetic variants and modification levels for two ARDS-associated CpGs (cg23344121, cg16212219). In summary, these ARDS-associated *MYLK* CpGs with effect modification by ethnicity and local mQTL, suggest that *MYLK* epigenetic variation and local genetic background may contribute to health disparities observed in ARDS.

Introduction

Acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome defined by increased vascular leakiness and pulmonary edema resulting from underlying inflammatory disease conditions. Despite improvements in supportive therapy in the management of ARDS, ARDS mortality remains at ~30-40% (1-8). Previous US epidemiologic studies identified a health disparity between persons of African descent (ADs) and European descent (EDs) in mortality from ARDS, with ADs displaying a higher mortality rate than EDs (9, 10). A study in the National Institutes of Health ARDS Network found higher mortality in persons of ADs than EDs before controlling for severity of illness at presentation (11). Additionally, a multicenter observational study found that ADs were less likely to develop the syndrome than EDs (12). These studies of differences in ARDS susceptibility and mortality between these and other ethnic groups (10-14), highlight the need for elucidating the underlying mechanisms of ARDS health disparities (15).

The search for implicated genes involved in ARDS initially focused on studying gene expression in various pre-clinical ARDS models and human case-control studies (16, 17). These studies, combined with pathway analysis of vascular barrier-regulatory genes and genome-wide association studies (GWAS) led to the selection of *MYLK* (encoding myosin light chain kinase; also known as MLCK) as an attractive ARDS candidate gene (18). Genetic studies have demonstrated that *MYLK* haplotypes confer ethnic-specific susceptibility to sepsis and sepsis-/trauma-induced ARDS (19-21). Functionally, MLCK regulates endothelial cell permeability via contractile pathways in response to inflammation (22-24). Genetic haplotypes may affect MLCK expression and/or functionality, thus providing a mechanism for ARDS susceptibility (25).

Specifically, the non-muscle isoform of MLCK (nmMLCK) has been implicated in increased lung injury in response to endotoxin administration both *in vitro* and *in vivo* (26-29). Furthermore, *MYLK* genetic variants in the form of single nucleotide polymorphisms (SNPs) were found to contribute to nmMLCK expression and/or protein function as expression quantitative trait loci (eQTL) (25, 30, 31). In addition to eQTL, epigenetic systems can also play a critical regulatory role in *MYLK* expression. However, the only *MYLK* epigenetic mechanism in ARDS explored to date involves microRNA-mediated regulation (32-34). Another form of epigenetic regulation, i.e. cytosine

modifications (primarily DNA methylation of cytosines at CpG dinucleotides) in *MYLK*, have not yet been evaluated for its potential role in the pathogenesis of ARDS. Interestingly, differentially modified cytosines in *MYLK* were recently identified in samples from healthy AD and ED individuals, thereby indicating significant baseline variation in methylation between these two populations (35, 36). Given the existence of natural genetic and epigenetic variations between individuals of ED and AD ancestry, understanding ethnicity-specific *MYLK* epigenetic regulation will enhance our knowledge of the molecular mechanisms underlying the observed ARDS disparities (37). In this study, we sought to first identify ARDS-associated epigenetic modification of *MYLK* by comparing cytosine methylation levels in ARDS patients with ICU controls; secondly find ethnicity-associated epigenetic variants among ARDS patients; and thirdly, to determine if common genetic variants contribute to the discovered epigenetic variations.

Materials and Methods

Human Subjects

A nested case control design was used to select whole blood DNA samples from two Chicago-based ARDS cohorts: Consortium for Investigating Intensive Care Unit Genetics (CIICUG, 2006 – 2009, University of Chicago) and Genomic Association Studies (GAS, 2010 – 2013, University of Illinois at Chicago). ARDS cases were defined by the 1994 American-European Consensus Conference (AECC) definition (38). Exclusion criteria in all patients included Hispanic heritage, diagnosis of cancer, history of organ transplant, or concurrent drug overdose, to maintain a clear AD/ED comparison and to exclude known confounders of cytosine modification (39). All samples were identified using a DNA identification number that was linked to demographic and clinical data, but not to personal identifiable data. Final distribution of cases and controls included 39 ARDS cases (18 EDs, 21 ADs) and 75 ICU controls (39 EDs, 36 ADs) for a total of 114 samples (Table 1).

Available data for all patients included: age, sex, and ethnicity. Clinical data available included presence of sepsis, septic shock, systemic inflammatory response syndrome (SIRS), and ICD-9 Codes (International Statistical Classification of Diseases and Related Health Problems). Data including weight and height, smoking status, whether the patient died in the ICU or in-hospital and APACHE II score (Acute Physiology and Chronic Health Evaluation II) were present for some but not all patients.

The study was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) and all sample handling and analyses included in this study was determined to not involve “human subjects” as defined in 45 CFR 46.102(f) by the Office for the Protection of Research Subjects at the University of Illinois at Chicago, where sample handling and analyses were based. The original Chicago-based cohort sample collections were approved by the Institutional Review Board at each participating institution, were also carried out according to The Declaration of Helsinki, and informed consent was obtained from all patients before blood draw.

DNA Sample Preparation and DNA Methylation Quantification

For the CIICUG cohort, DNA was isolated from individual patient's whole blood using the FlexiGene DNA Kit (QIAGEN) according to company protocol and subsequently maintained in −20 to −80°C freezers. For the GAS cohort, DNA was isolated from individual patient's whole blood using the iPrep PureLink gDNA Blood Kit (Invitrogen) according to company protocol and subsequently maintained in −20 to −80°C freezers. For each selected sample, 800ng DNA was submitted for bisulfite conversion (EZ-96 DNA Methylation Kit, Zymo Research). The efficacy of the bisulfite conversion was checked by PCR amplification and DNA sequencing of selected genomic regions. Approximately 150 ng of bisulfite-converted DNA from each sample was used for profiling DNA methylation levels using the Illumina Infinium Human Methylation 450 BeadChip (450K array) (40), according to the manufacturers' protocols at the University of Chicago Genomics Core.

DNA Methylation Data Processing

The 450K array data were processed according to a previous a publication (35). Briefly, the M-values ($\log_2$ ratio of the intensities of modified probe versus unmodified probe) (41) of CpG probes that passed quality control based on detection p-value ($p<0.01$) across at least 95% of samples were summarized and batch corrected using COMBAT (42). We also detected 15 CpG probes in *MYLK* that are mapped to multiple loci in the human genome (43) or contain known common genetic variants based on the dbSNP v135 (44). The final test set was comprised of 37 CpG sites located within the *MYLK* gene. The genomic location groups (e.g., promoter, gene body) were assigned according to the annotations provided by Illumina. The raw and processed 450K array data have been deposited into NCBI Gene Expression Omnibus database (accession number: GSE67530).

Statistical Analysis

Data analysis was performed with SAS 9.4 (Cary, NC) with a dichotomous dependent variable ARDS case or ICU control. Characteristics of the patient and control groups were compared using one-way ANOVA, Wilcoxon-Mann-Whitney, Chi-square or Fisher's exact tests, as appropriate (Table 1). Normally distributed M-values (logit transformation of the β-value, i.e., the ratio of methylated probe intensity and overall intensity) were used (41). Logistic regression modeling with ARDS status as the dependent variable was used to assess the association of M-values of individual CpGs and potential effect modification by age (continuous), sex and ethnicity by including interaction terms. For CpGs with significant ($p<0.10$) ethnicity*M-value or age*M-value interaction terms, analysis was stratified by ethnicity or age (<58 years of age and ≥58 years of age categories) as appropriate. Sex, age and ethnicity were retained as covariates if the model was not already stratified by one of these variables. Including these covariates improved the fit of the model, as analyzed by the log likelihood when adding or removing terms and the Hosmer-Lemeshow Goodness-of-Fit Test, and it is consistent clinically to control for these factors (45-47). Effect modification was determined to be present if the interaction term was statistically significant in a logistic regression model and if the directionality or magnitude of association differed substantially. A nominal p-value of < 0.05 was utilized to identify differential CpGs, and the false discovery rate (FDR) was controlled by q-value for the crude unadjusted associations (48).

Some crude unadjusted associations did not meet the nominal p or FDR, however stratified associations met the nominal p-value of < 0.05 requirement (Table 2).

Mapping of mQTL

Genotyping was performed on some of the DNA samples from our on-going GWAS (unpublished data) using the Affymetrix Axiom Genome-Wide PanAFR (Pan-African array) and Affymetrix Genome-Wide Human SNP Array 6.0 according to the manufacturer's recommendation at the University of Illinois DNA Services Facility. SNPs 1Mb up-/down-stream from the *MYLK* gene were imputed to the 1000 Genomes Project (49) Phase I integrated data using IMPUTE2 (50). Both directly genotyped and imputed SNPs with MAF (minor allele frequency) > 0.01 and missing rate < 25% (314 local SNPs, in total) were retained for mQTL mapping. Given that mQTL are predominantly local to target CpGs (39), we included SNPs within 1Mb of target CpGs in *MYLK* to map *cis*-acting (local) mQTL associated with the CpG modification levels using a linear regression model with sex as a covariate. FDR for mQTL mapping was controlled using the Benjamini-Hochberg (BH) approach (51).

Results

Comparisons of General Characteristics, Clinical Characteristics and Comorbidities

ARDS cases and ICU controls had similar distributions of age and sex (Table 1). Clinical indices, including APACHE II scores and other variables associated with severity, were not statistically different between ARDS cases and ICU controls, except for occurrence of death during the current ICU or hospital stay (Died ICU/Hosp.) where ARDS cases where the odds of dying was higher during the current stay compared to ICU controls (OR = 3.1, 95% CI 1.22-7.82, p = 0.014). Presence of comorbidities was also not statistically different between cases and controls except for pneumonia, being more common among ARDS cases; endocrine disease, being more common among ICU controls; and obesity, being less common among AD ARDS cases (Table 1).

Identification of CpGs in *MYLK* Associated with ARDS

In logistic regression models, of the 33 unambiguous, high quality CpG probes located within *MYLK* for which ethnicity or age were found not to be an effect modifier for methylation level, two CpG probes, cg03892735 (intron 4,5) and cg23344121 (exon 18) were found to be significantly associated with ARDS status ($p<0.05$; $q<0.25$, Table 2, Figure 1B). One CpG, cg19611163 (intron 19,20), for which age was found to be an effect modifier was analyzed in stratified logistic regression models and was found to be differentially methylated between ARDS patients and ICU controls among those less than 58 years of age, but not those 58 years or older, where patients less than 58 years of age were less methylated in ARDS cases compared to ICU controls (Table 2, Figure 1D). Two CpGs for which ethnicity was found to be an effect modifier were evaluated in stratified logistic regression models and were found to be differentially methylated between ARDS subjects and ICU controls among EDs, but not ADs (Table 2, Figure 1C). No modifications unique to AD ARDS cases were found. Of the *MYLK* cytosines modified in ED ARDS cases, cg01894985

(intron 2,3) and cg16212219 (intron 31,32) were more methylated in ARDS cases compared to ICU controls (Figure 1A, 1C).

Mapping of mQTL for ARDS-associated CpGs in *MYLK*

To assess the potential effect of genetic variation on cytosine modification, mQTL were mapped for the ARDS-associated and ethnicity-interacting CpGs in *MYLK* (Figure 1A). At the FDR corrected $p < 0.05$, there were six local SNPs (rs7650050, rs7630208, rs7652791, rs6780229, rs61281424, and rs59204624) (Table 3) significantly associated with cg23344121 ($p = 6.84e\text{-}5$, $r^2 = 0.23$). All six SNPs were located in a linkage disequilibrium (LD) block ($r^2=1$). Eight local mQTL were detected for cg16212219 with a BH-adjusted p-value of <0.05 (Figure 2, Table 3). For other tested CpGs: cg01894985, cg03892735 and cg19611163 no local mQTL was detected at a BH-adjusted $p < 0.10$.

Discussion

This study identified two epigenetic variants, cg03892735 (intron 4,5), cg23344121 (exon 18), associated with ARDS in both ethnicities, one CpG site associated with ARDS only among those less than 58 years of age, and two CpG sites associated with ARDS only among EDs, when controlling for sex, age and/or ethnicity as appropriate. The two *MYLK* CpGs associated with ARDS in EDs were more methylated (cg01894985 and cg16212219, located in the gene body) in ARDS cases compared with ICU controls. Of these two CpG sites, gene body cg16212219 was found to have eight associated local SNPs, indicating possible contribution of local genetic variation to this epigenetic variant. Although we hypothesize that the observed variations in CpG modification may result in altered *MYLK* expression, future studies are necessary to test this hypothesis.

ARDS is a devastating clinical syndrome with a substantial mortality, annually affecting an estimated 190,600 people and attributing to 3.6 million hospital days in the United States (1). Although AD patients display a higher mortality rate than ED patients even after adjusting for demographic and clinical variables (9-11), in the current study, significant differences were not identified for in-hospital mortality between AD and ED ARDS patients. These results highlight several limitations of the study including the limited sample size due to the number of available samples and the applied exclusion criteria. Socioeconomic data and cause of death were not available. Height and weight and APACHE II score were also not available for all patients; therefore, it was not feasible to enter BMI or APACHE II scores into the final regression analyses as it would dramatically reduce the number of samples analyzed. DNA was obtained from peripheral whole blood with multiple cell types, and complete blood cell counts were not available. As multiple physicians phenotyped the ARDS cases, there is a chance for misclassification of cases (52), although the 1994 AECC definition was used in chart evaluation. Type I error is possible due to the false discovery rate of 25% and alpha threshold of 0.05 over 37 CpG sites evaluated. This analysis was not performed in a replicate manner, and it would be of benefit to reproduce these data in another cohort.

While the functionality of the differentially modified CpGs identified in this study needs to be further studied, it is evident that there are novel epigenetic differences in the *MYLK*

gene, associated with genetic variation and modified by ethnicity, between ARDS cases and ICU controls. Understanding these differences and further elucidating the effect of the specific SNPs and CpG modification levels on gene expression will be key next steps in mechanistically understanding the genetic-epigenetic effects on health disparities in ARDS.

Acknowledgements

This work was supported by USDA/NIFA grant AG 2010-34283-21243 (K.L.S., W.Z), NIH grants HG006367 (W.Z), HL 91889 (J.G.N.G.), HL 125615 (J.G.N.G.); P01 HL126609 (J.G.N.G.), and OD010914 (J.D.F.). The authors would like to thank Dr. Rick Kittles for valuable discussions and Michael Wade for his work in sample preparation.

Abbreviations

ARDS	(acute respiratory distress syndrome)
AD	(African descent)
ED	(European descent)
MLCK	(myosin light chain kinase protein)
MYLK	(myosin light chain kinase gene)
CpG	(cytosine-guanine dinucleotide)
ICU	(intensive care unit)
mQTL	(modified cytosine quantitative trait loci)
BMI	(body mass index)
APACHE II	(Acute Physiology and Chronic Health Evaluation)
OR	(odds ratio)

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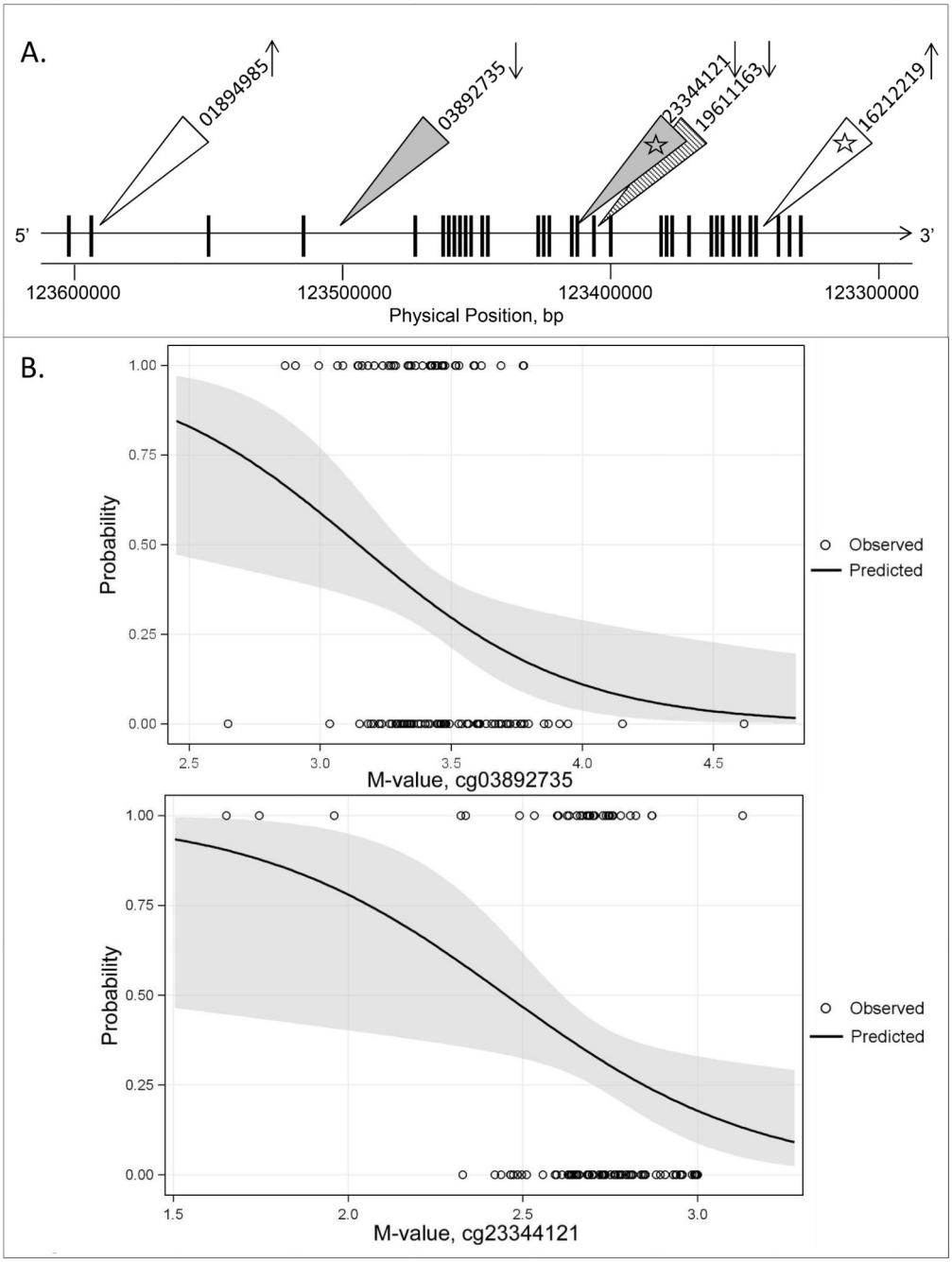
Brief Commentary

Background

Acute respiratory distress syndrome (ARDS) is accompanied with substantial mortality, especially in African descent patients. Despite recent advances, underlying mechanisms influencing ethnic health disparities in ARDS are not fully understood.

Translational Significance

Our nested case-control study found epigenetic modification variation of the well-described ARDS candidate gene, *MYLK* encoding myosin light chain kinase to be associated with ARDS patients and European ancestry. Epigenetic differences were partially associated with local genetic variation as well. Understanding the mechanisms of these ethnicity-specific and ARDS-associated modifications will be the next step in illuminating the genetic-epigenetic influences on health disparities in ARDS.



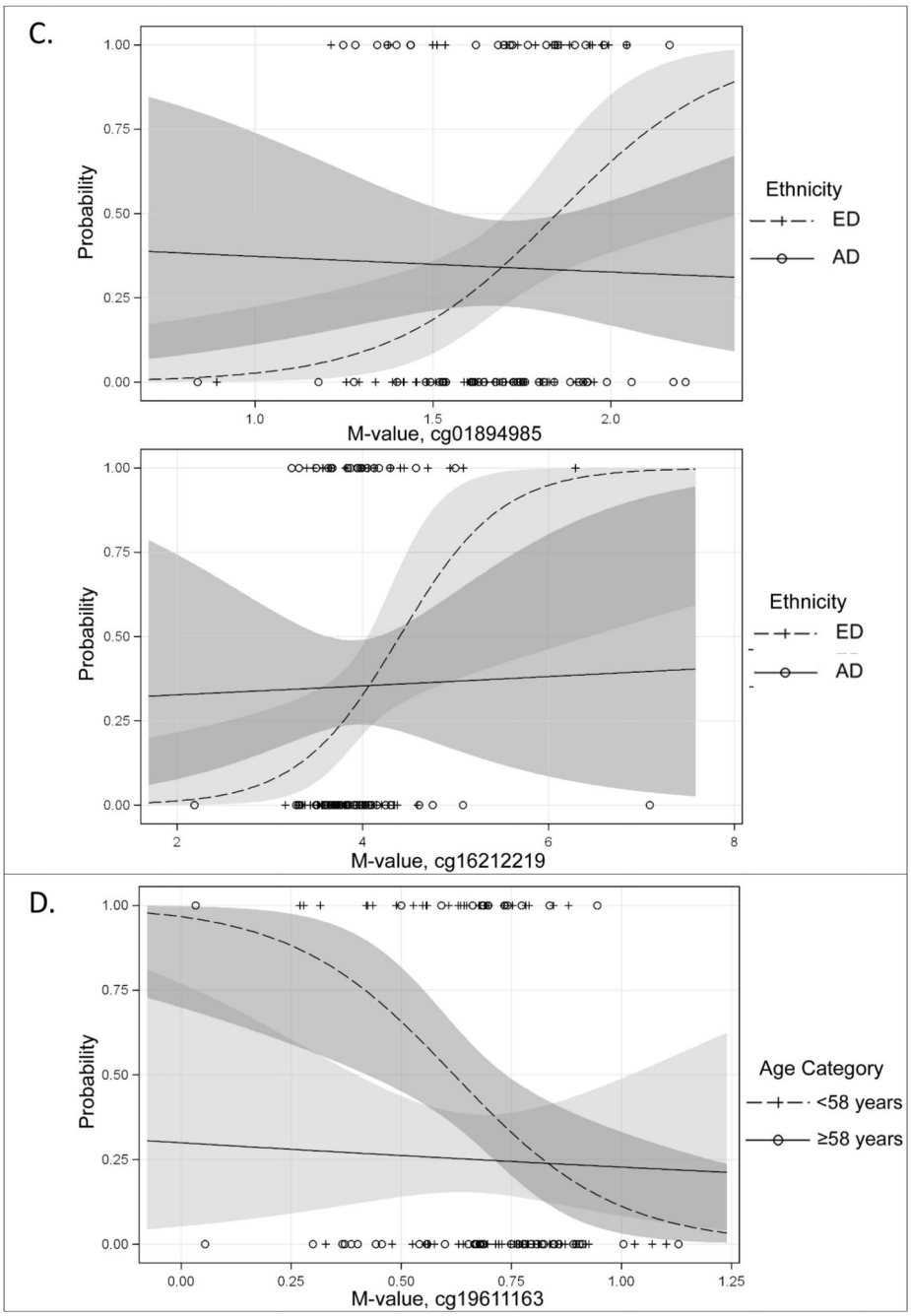


Figure 1. ARDS-associated and population-modified CpGs in *MYLK*

(A). Locations of significant CpGs after controlling for age and sex and stratifying by ethnicity (cg01894985, cg16212219), for age and ethnicity and stratifying by age (cg19611163), and for age, ethnicity and age (cg03892735, cg23344121), in logistic regression are indicated based on hg19. Vertical bars are exons; white triangles indicate significantly differentially modified CpG sites for ED ARDS cases vs. ED ICU controls; striped triangle indicates a significant CpG for less than 58 year old ARDS vs. less than 58 year old ICU controls; light gray triangles indicate significant CpG sites for ARDS cases vs.

ICU controls with no effect modification by age or ethnicity; arrows indicate higher modification (up arrow) or lower modification (down arrow) for ARDS cases vs. ICU controls; numbers correlate to CpG probe ID numbers in the 450k array; white stars indicate significant mQTL detected for two target CpG sites.

(B). Significantly modified CpG sites for ARDS cases vs. ICU controls controlling for age, sex and ethnicity for which no significant M-value interaction terms were identified: cg03892735, cg23344121, located in intron (4,5) and exon 18, respectively. Effect plots of the probability that a case has ARDS (y-axis) according to M-value (x-axis). Open circles indicate observed case status (1 = ARDS, 0 = ICU control), line the predicted probability across M-value, shaded area the 95% confidence limits for the predicted probabilities.

(C). Significantly modified CpG sites for ARDS cases vs. ICU controls among ED, controlling for age and sex: cg01894985, cg16212219, located in intron (2,3) and intron (31,32) respectively. Effect plots of the probability that a case has ARDS (y-axis) according to M-value (x-axis). Plus signs indicate observed ED case status and open circles observed AD case status (1 = ARDS, 0 = ICU control), predicted probability across M-value is indicated by a dashed line for ED patients and a solid line for AD patients, and the shaded areas (light gray for ED, dark gray for AD) indicate the 95% confidence limits for the predicted probabilities.

(D). Significantly modified CpG site for ARDS cases vs. ICU controls among those less than 58 years of age, controlling for sex and ethnicity: cg19611163, located in intron (19,20). Effect plot of the probability that a case has ARDS (y-axis) according to M-value (x-axis). Plus signs indicate observed <58 year old patient case status, open circles indicate observed ≥58 year old patient case status (1 = ARDS, 0 = ICU control), predicted probability across M-value is indicated by a dashed line for <58 year old patients and a solid line for ≥58 year old patients, and the shaded areas (dark gray for <58 year, light gray for ≥ 58 year) indicate the 95% confidence limits for the predicted probabilities.

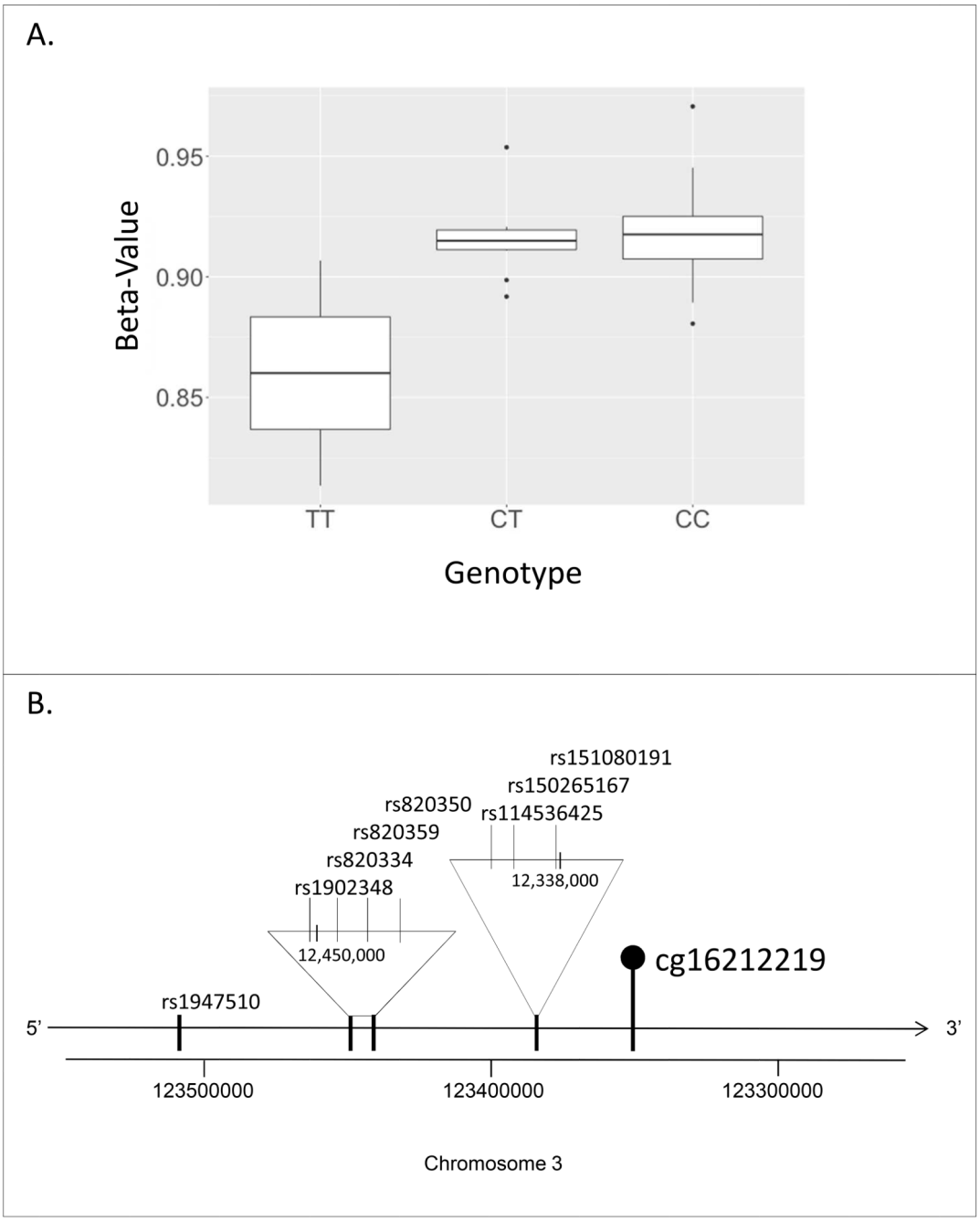


Figure 2. mQTL mapping for an ARDS-associated CpG in *MYLK*

(A). Box and whisker plots show the beta-values of cg16212219 and genotypes of one of its local mQTL, rs820359. The first and third quartiles are indicated at box ends, median by the horizontal line, the minimum and maximum by the end of the whiskers, and outliers by the dots.

(B). The physical positions (hg19) of cg16212219 and its eight detected local mQTL on chromosome 3 are shown.

Table 1

General characteristics, clinical indices and comorbidities of the study population

	African Descent			European Descent		
	ARDS Cases (n = 21)	**ICU Controls (n = 36)**	***p***	**ARDS Cases (n = 18)**	**ICU Controls (n = 39)**	***p***
Age, mean yrs (+/−SD)	49 (+/−17.4)	55 (+/−20.6)	0.26 *	56 (+/−17.6)	61 (+/−15.7)	0.27 *
Male, n (%)	12 (57%)	18 (50%)	0.60 †	11 (61%)	24 (62%)	0.98 †
APACHE II, (25-75%) (n available)	22 (18-28) (n = 14)	25 (21-29) (n = 27)	0.45 ‡	21 (19-36) (n = 7)	22 (15-26) (n = 25)	0.39 ‡
SIRS, n (%)	17 (81%)	34 (94%)	0.18 §	12 (71%)	33 (85%)	0.28 §
Sepsis, n (%)	17 (81%)	33 (94%)	018 §	13 (76%)	25 (64%)	0.36 †
Septic shock, n (%)	12 (60%)	21 (60%)	1.00 †	9 (53%)	17 (44%)	0.52 †
Died ICU/Hosp, n (%)	7 (35%)	5 (16%)	018 §	8 (47%)	6 (20%)	0.05 †
Obese, n (%)	5 (29%)	16 (62%)	**0.04** †	5 (63%)	16 (64%)	1.00 §
Lung disease, n (%)	21 (100%)	28 (78%)	**0.02** §	18 (100%)	20 (51%)	**<.001** §
Pneumonia	12 (57%)	9 (25%)	**0.02** †	11 (65%)	5 (13%)	**<.001** §
Endocrine, n (%)	4 (19%)	18 (50%)	**0.02** †	5 (29%)	23 (59%)	**0.04** †
Diabetes mellitus	4 (19%)	12 (33%)	0.25 †	4 (24%)	21 (54%)	**0.04** †
Hypertension, n (%)	10 (48%)	20 (56%)	0.56 †	9 (53%)	19 (49%)	0.77 †
Cardiac disease, n (%)	10 (48%)	19 (53%)	0.71 †	7 (41%)	24 (52%)	0.16 †
Renal disease, n (%)	11 (52%)	26 (72%)	0.12 †	9 (53%)	21 (54%)	0.95 †
Rheumatologic, n (%)	5 (24%)	10 (28%)	0.74 †	2 (12%)	8 (21%)	0.43 †
Smoker, n (%)	10 (63%)	15 (65%)	0.86 †	8 (80%)	15 (60%)	0.43 §

*One-way ANOVA,

†Chi-square,

‡Wilcoxon-Mann-Whitney median (25-75%),

§Fisher's exact are the associated statistical tests for each analysis between ARDS cases and ICU controls within AD and ED individuals as indicated on each row. There were no significant differences found between AD and ED ARDS cases. Percentages represent the percent subjects with the condition out of the total number of subjects with available data within each group.

Table 2

M-values: Differentially modified *MYLK* CpGs, with M-value as the main effect, in logistic regression with ICU controls as the reference for the dependent variable, adjusting for: age, sex and ethnicity (cg03892735, cg23344121); age and sex (cg01894985, cg16212219); sex and ethnicity (cg19611163). Adj. = adjusted odds ratio, Cr. = crude unadjusted odds ratio, AD and ED or <58 yrs and ≥58 yrs = stratified odds ratios, indicating effect modification by ethnicity or age. β = regression coefficient, SE = standard error.

CpG Location Base Position		Odds Ratio	95% CI	β	SE	p
cg01894985	Cr.	3.01	0.57, 15.76	1.10	0.84	0.192
Intron (2,3)	Adj.	3.98	0.74, 21.52	1.38	0.86	0.109
123589179	AD	0.81	0.10, 6.48	−0.21	1.06	0.845
	ED	*205	4.50, >999	5.32	1.95	0.006
cg03892735	Cr.	*0.09	0.01, 0.55	−2.44	0.94	0.009
Intron (4,5)	Adj.	*0.09	0.01, 0.56	−2.45	0.95	0.010
123506296						
cg23344121	Cr.	*0.07	0.008, 0.59	−2.67	1.09	0.015
Exon 18	Adj.	*0.06	0.006, 0.63	−2.79	1.19	0.018
123419622						
cg19611163	Cr.	*0.10	0.01, 0.78	−2.32	1.06	0.028
Intron (19,20)	Adj.	*0.09	0.01, 0.75	−2.40	1.08	0.026
123411211	<58 yrs	*0.03	0.001, 0.58	−3.53	1.53	0.021
	≥58 yrs	0.19	0.007, 4.96	−1.65	1.66	0.320
cg16212219	Cr.	1.94	0.92, 4.07	0.66	0.38	0.082
Intron (31,32)	Adj.	1.91	0.92, 3.99	0.65	0.37	0.083
123339918	AD	1.07	0.44, 2.57	0.06	0.45	0.889
	ED	*6.14	1.37, 27.58	1.81	0.77	0.018

*odds ratio with a nominal p value <0.05.

Table 3

Beta-values: Six and eight mQTL detected for cg23344121 and cg16212219, respectively. β = regression coefficient, SE= standard error, r^2 = correlation coefficient, p = unadjusted p-value, BH-p = Benjamini-Hochberg-adjusted p-value.

SNP	β	SE	r^2	p	BH-p
cg23344121					
rs112200373	−0.0191	0.0044	0.2318	5.6E-05	0.0031
rs59204624	−0.0191	0.0045	0.2305	6.8E-05	0.0031
rs61281424	−0.0191	0.0045	0.2305	6.8E-05	0.0031
rs6780229	−0.0191	0.0045	0.2305	6.8E-05	0.0031
rs7652791	−0.0191	0.0045	0.2305	6.8E-05	0.0031
rs7630208	−0.0191	0.0045	0.2305	6.8E-05	0.0031
SNP	**β**	**SE**	**r^2**	**p**	**BH-p**
cg16212219					
rs820350	−0.0225	0.0063	0.1676	0.0006	0.0037
rs820334	−0.0163	0.0048	0.1287	0.0012	0.0037
rs1902348	−0.0173	0.0052	0.1298	0.0014	0.0037
rs151080191	−0.0231	0.0079	0.1095	0.0045	0.0080
rs150265167	−0.0234	0.0081	0.1133	0.0054	0.0080
rs114536425	−0.0234	0.0083	0.1129	0.0062	0.0080
rs1947510	−0.0135	0.0048	0.1169	0.0070	0.0080
rs820359	−0.0130	0.0048	0.0936	0.0080	0.0080