ORIGINAL ARTICLE

Epigenetic contribution of the myosin light chain kinase gene to the risk for acute respiratory distress syndrome

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Acute respiratory distress syndrome (ARDS) is a devastating clinical syndrome with a considerable case fatality rate (~30%–40%). Health disparities exist with African descent (AD) subjects exhibiting greater mortality than European descent (ED) individuals. Myosin light chain kinase is encoded by *MYLK*, whose genetic variants are implicated in ARDS pathogenesis and may influence ARDS mortality. As baseline population-specific epigenetic changes, that is, 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* cytosine-guanine dinucleotides (CpGs) between ARDS patients and intensive care unit (ICU) controls overall and by ethnicity in a nested case–control study of 39 ARDS cases and 75 non-ARDS ICU controls. Two *MYLK* CpG sites (cg03892735 and cg23344121) were differentially modified between ARDS subjects and controls ($P < 0.05$; $q < 0.25$) in a logistic regression model, where no effect modification by ethnicity or age was found. One CpG site was associated with ARDS in patients aged <58 years, 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 modified cytosine quantitative trait loci (mQTL) were identified using linear regression between local genetic variants and modification levels for 2 ARDS-associated CpGs (cg23344121 and 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. (Translational Research 2016;■:1–10)

Abbreviations: AD = African descent; APACHE II = acute physiology and chronic health evaluation II; ARDS = acute respiratory distress syndrome; CpG = cytosine-guanine dinucleotide; ED = European descent; ICU = intensive care unit; mQTL = modified cytosine quantitative trait loci; MLCK = myosin light chain kinase protein; *MYLK* = myosin light chain kinase gene

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AT A GLANCE COMMENTARY

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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.

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 (AD) and European descent (ED) 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 AD than ED before controlling for severity of illness at presentation.[11] In addition, 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 preclinical 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 led to the selection of *MYLK* (encoding myosin light chain kinase [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, Q4 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 Q5 ARDS susceptibility.[25] Specifically, the nonmuscle isoform of MLCK 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 nonmuscle isoform of MLCK expression and/or protein function as expression quantitative trait loci.[25,30,31] In addition to expression quantitative trait loci, 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, that is, cytosine modifications (primarily DNA methylation of cytosines at cytosine-guanine dinucleotides [CpGs]) in *MYLK*, has 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 a significant baseline variation in methylation between these 2 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 first sought to identify ARDS-associated epigenetic modification of *MYLK* by comparing cytosine methylation levels in ARDS patients with intensive care unit (ICU) controls; second, to find ethnicity-associated epigenetic variants among ARDS patients; and third, 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 2 Chicago-based ARDS cohorts: Consortium for Investigating Intensive Care Unit Genetics (2006–2009, University of Chicago) and Genomic Association Studies (2010–2013, University of Illinois at Chicago). ARDS cases were defined by the 1994 American-European Consensus Conference