

Exploring DNA Methylation of *MYLK* as a Contributor to Acute Respiratory Distress Syndrome Disparities

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Acute Respiratory Distress Syndrome (ARDS) affects approximately 200,000 people annually in the United States with a substantial mortality rate at ~40% [1]. The epidemiology of ARDS is defined by ethnicity, sex, age and modifiable risk factors such as smoking history and alcohol use. Specifically, the mortality rate among persons of African Descent (AD) with ARDS is significantly higher than persons of European Descent (ED) with ARDS, after controlling for socioeconomic status and access to healthcare, indicating a potential genetic influence on outcome [2,3]. However, after adjusting for severity of illness this association disappears in some studies [3,4], and AD individuals might be protected against the development of ARDS in trauma cases [4]. There has been extensive research exploring the underlying mechanisms of these health disparities in ARDS, but the specific processes remain unclear.

Previous studies using the candidate gene approach have implicated genes involving endothelial and epithelial permeability in ARDS. One comprehensively studied candidate gene is *MYLK* (encoding myosin light chain kinase or MLCK), an isoform (nmMLCK: non-smooth muscle MLCK) involved in vascular endothelial cell gap formation and vascular leakage, as well as implicated in the inflammatory responses including apoptosis and leukocyte diapedesis [5,6]. The results from both *in vivo* [7-10], *ex vivo* [11,12] and *in vitro* [13,14] studies provide solid evidence for the role of nmMLCK in the pathogenicity and susceptibility of ARDS. In order to investigate whether the genetic variants in *MYLK* might contribute to the health disparities associated with ARDS, several case-control studies have been performed to compare AD and ED populations. In particular, population-specific SNPs (single nucleotide polymorphisms) in *MYLK* exist between AD and ED individuals with ARDS, as well as severe asthma [15], suggesting a potential genetic contribution to lung health disparities [16,17]. The precise function of these genetic variants is unknown, however the knowledge of these variations allow for further research into the mechanisms of ARDS and relevant disease processes. For example, specific SNPs located in the promoter region of *MYLK* are associated with *MYLK* expression [18], thus potentially contributing to the health disparities through the differential allele frequency of the functional allele in a particular population. However, gene expression is such a complex trait or phenotype that is controlled by various genetic and non-genetic factors. More recently, epigenetic mechanisms including DNA methylation and histone modifications have begun to be investigated for their roles in regulating quantitative gene expression. A comprehensive investigation of these epigenetic mechanisms for their relationships with *MYLK* expression and their variation between human populations may provide novel insights into the underlying genetic basis of the health disparities in ARDS.

One highly viable epigenetic mechanism is DNA methylation, which is covalent modification typically at CpG dinucleotides across the human genomes. It has been found that silenced genes often have promoter regions with greater numbers of methylated cytosines than actively transcribed genes, suggesting that DNA methylation is a factor in transcriptional repression [19,20]. DNA methylation is also found in gene bodies, which may represent additional gene regulatory locations, possibly through regulating chromatin structure and

accessibility of transcription machinery [21-23]. DNA methylation is a stable, mitotically inheritable trait that is maintained by DNA methyltransferase (DNMT1) and new methylation occurs mainly via DNA methylation enzymes DNMT3A, DNMT3B and DNMT3L [24]. Utilizing high throughput microarray technologies, recent studies have demonstrated significant natural variation in DNA methylation between human populations. For example, a previous study using the Illumina Infinium HumanMethylation 27K array (covering ~27,000 promoter CpGs) reported a substantial proportion (~30%) of population-specific CpGs between individuals of African and European ancestry [25].

More recently, the availability of the Illumina 450K array (covering more than 450,000 CpGs) has allowed a much more comprehensive scan of the human genome (covering promoter regions, gene bodies, Untranslated regions [UTRs], and intergenic regions) for population variation in DNA methylation using the HapMap CEU (Caucasian residents from Utah, USA) and YRI (Yoruba people from Ibadan, Nigeria) samples [23]. Similar to Fraser et al, a substantial proportion of CpG sites (13%) showed population-specific DNA methylation levels at a False Discovery Rate (FDR) of 1% using the 450K array data [23]. Particularly, significant cytosine modification variation in *MYLK* was identified between the CEU and YRI samples using the 450K array data. Out of the 52 total CpGs profiled on the 450K array for *MYLK*, eight CpGs were found to differentially methylated between individuals of African and European ancestry at FDR<1%. Furthermore, local (e.g., within 100kb) SNPs were identified to be associated with all eight population-specific CpGs, suggesting that the underlying genetic variation may contribute to the cytosine modification variation in *MYLK*. For example, the allele C of an intronic SNP in *MYLK* (rs6438808) was associated with a gene-body CpG (Illumina probe ID: cg12235788) in *MYLK*. Understanding the potential contribution of these population-specific CpGs in ARDS patients may advance the interpretation of factors that contribute to the health disparities observed in ARDS.

In summary, *MYLK*, a candidate gene involving endothelial and epithelial permeability is a critical participant in the pathogenesis of ARDS. The genetic variation in *MYLK* may account for the observed health disparities in ARDS between human populations. Technical advances have allowed exploring further gene regulatory mechanisms, such as DNA methylation variation in *MYLK* among patients from different populations, thus enhancing our knowledge of the underlying mechanism for ethnical disparities in ARDS.

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