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Myosin light chain kinase gene and acute lung injury in trauma and sepsis: Opposite effects but confirmatory

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) usually develops in response to a major insult such as sepsis, trauma, pneumonia, and multiple transfusions (1). Our current understanding of why some patients develop and die of ALI although others do not is incomplete. Recently, discoveries about the genetic control and regulation of innate immunity and inflammatory response have raised the question of whether the multiple polymorphic alleles of genes that encode for cytokines and other mediators of inflammation may result in phenotypic differences in host inflammatory response to an inciting injury. Although genetic determinants to the development of and outcome in ALI/ARDS have only recently been considered and studied, there has been a sudden explosion of studies in this field in the last few years.

In this issue of *Critical Care Medicine*, Christie et al. (2) make the latest contribution to this growing field with their study on the *myosin light chain kinase (MYLK)* gene and susceptibility to developing ALI in patients with major trauma. Their study has a number of notable strengths. First, the prospective cohort design and the daily screening of all patients are less susceptible to selection bias and misclassification of ALI. All non-ARDS patients are appropriately derived from an at-risk population. ALI is well phenotyped with careful attention to minimizing the uncertainties of the American European Consensus criteria. The selection of *MYLK* as a candidate gene for investigation is founded on sound evidence from functional genomic studies in various animal models of ALI and on a prior association found between variations in the *MYLK* gene and development of sepsis and sepsis-induced ALI.

So what are we to make of the results from Dr. Christie and his colleagues? In their earlier study of patients with sepsis, the authors found that the A allele at rs28497577 and the C allele at the rs9840993 were associated with increased risk of developing ALI (3). In contrast, Dr. Christie and colleagues found that these alleles and their associated haplotypes were protective against development of ALI in trauma. Could such opposite findings arise from chance and hence, be a false positive finding? Using a Bayesian or semi-Bayesian approach which incorporates the prior probability of finding an association that is inherent in the selection of candidate genes for investigation, one can calculate the false positive report probability for the associations reported by Dr. Christie et al (4). In this study, if one assumes a high prior

probability of 0.2 for detecting an effect (given that *MYLK* was chosen on the basis of functional genomic studies and a prior association with ALI and sepsis), then the odds ratio for rs28497577 would have a false positive report probability <0.37, below the threshold of 0.5 considered by Wacholder et al. (4) to be noteworthy for small genetic association studies like these. This is significantly higher than the false positive report probability for most of the genetic susceptibility studies in sepsis (5).

The opposite effect from the same single nucleotide polymorphism (SNP) is not necessarily contradictory. Some have argued that the replication of genetic association studies should be viewed at the level of the gene rather than a specific variant, haplotype, or effect in different populations (6). Indeed, many of the most well replicated asthma susceptibility genes are based on an association with different functional variants within the same gene or opposing alleles at the same SNP in different populations (7). As such, Dr. Christie and colleagues' report should still be considered confirmatory for a role of the *MYLK* gene in the development of ALI.

The opposite effect estimate for the *MYLK* gene and ALI/ARDS in trauma and sepsis indicates the possibility of effect modification by the type of risk factor predisposing to ALI and the effect of *MYLK* genotype on ALI/ARDS susceptibility. Such gene-environment interactions from the injury predisposing to lung injury have been found previously for the C allele of +1580CT polymorphism in the *surfactant protein-B* gene and the -308GA polymorphisms in the *tumor necrosis factor- α* gene (8,9). Defining these gene-environment interactions is important. Many of the polymorphisms identified in ARDS are common with frequency greater than 1%. Given its persistence in the genome and the lethality of ARDS, it is unlikely that these polymorphisms are universally detrimental. Rather, it is likely that these variants may be detrimental in some situations and benign or even beneficial in others.

It is not clear why *MYLK* may behave differently in trauma vs. sepsis. The cytokine profile and inflammatory markers between ARDS patients and at-risk non-ARDS patients differ depending on whether their predisposing injury was sepsis, trauma, acute pancreatitis, or massive transfusion (10,11). *MYLK* have a variety of functions including regulation of vascular permeability, apoptosis, leukocyte diapedesis, and activation of myosin and cytoskeleton which can influence bronchial contractility and clot retraction in hemostasis (12–14). One can easily postulate that the dominant function of *MYLK* at any one time would depend on whether the environmental stressor was inflammatory from infection or bleeding from trauma. However, one limitation in the study from Dr. Christie and colleagues is the assumption that trauma is a homogeneous risk factor for ALI. Trauma from gunshot wounds is likely to result in massive transfusion which may have very different mechanisms for lung injury than blunt trauma or pulmonary contusions. Because the functional significance of the at-risk polymorphisms and haplotypes are beyond the scope and aim of this study, it is not clear how *MYLK* may differ in sepsis vs. trauma and in different types of trauma. Additional studies under different environmental insults will be needed to better define the role of *MYLK* in ALI.

Another notable feature of Dr. Christie and his colleagues' study is the large numbers of both African Americans and European Americans in their cohort which allowed for examination of racial differences in the genetic susceptibility to developing ALI. African Americans have been demonstrated to have a higher incidence of ARDS mortality than whites and Hispanics (15). Some have raised questions about whether genetic variability may explain such racial differences in ALI/ARDS. In the article by Christie et al., the association between *MYLK* SNPs and haplotypes and ALI was stronger among African Americans. The effect of a SNP may be greater in one racial group for several reasons. The examined SNP may be in linkage disequilibrium with the disease locus and the linkage may be tighter in certain ethnic groups than others. There may be modifying genes that interact with *MYLK* or there may be multiple

susceptibility loci within or near the gene that may have different frequencies in different populations. However, it is not clear that the racial differences found in this study can be attributed to any of these reasons. There were important differences between the African Americans and European Americans at baseline in the study which may confound the issue of race. African Americans in the cohort were younger, more likely male, with lower frequency of blunt trauma and required more transfusion than European Americans. Such differences make it difficult to determine whether there are genuine differences in the effect of the *MYLK* gene in different racial groups. Large cohorts of patients from well-defined racial groups will be needed to better define racial differences in the genetic susceptibility to developing ALI.

With the development of pathway based approaches and genome-wide association studies, we will soon be able to examine large numbers of genes rapidly and not be limited to known genes. Although large cohorts of patients with ALI are accumulating, the high mortality seen in ALI limits the number of prevalent cases and it is doubtful that in a short period of time, sample sizes in ALI will rival the large studies of other complex genetic diseases like diabetes or breast cancer. Our ability to make meaningful sense of such large volumes of data with a limited number of ALI patients will depend on advances in bioinformatics and the precision and accuracy of the phenotype of the patients. Regardless of the approach, more confirmatory studies of genetic associations with ALI susceptibility and outcomes will be needed. These studies need to be both sufficiently powered and clearly phenotyped, both in terms of the classification of ALI and in the precipitating injury leading to ALI. Likewise, future animal investigations in ALI should consider employing a different mechanism of lung injury to better examine potential gene-environment interactions. Ultimately, the likelihood of a cause and effect relationship underlying any genetic association will depend on the reproducibility of well-designed studies in different populations and in the strength of the biological rationale behind the selection of that gene for analysis.

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