Genetic Determinants and Ethnic Disparities in Sepsis-associated Acute Lung Injury

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Acute lung injury (ALI) is a common and devastating illness that occurs in the context of sepsis and other systemic inflammatory disorders. In systemic illnesses like sepsis, only a subset of patients develops ALI even when pathologic stimuli are apparently equivalent, suggesting that there are genetic features that may influence its onset. Considerable obstacles in defining the exact nature of the pathogenesis of ALI include substantial phenotypic variance, incomplete penetrance, complex gene-environment interactions and a strong potential for locus heterogeneity. Moreover, ALI arises in a critically ill population with diverse precipitating factors and appropriate controls that best match the reference population have not been agreed upon. The sporadic nature of ALI precludes conventional approaches such as linkage mapping for the elucidation of candidate genes, but tremendous progress has been made in combining robust, genomic tools such as high-throughput, expression profiling with case-control association studies in well characterized populations. Similar to trends observed in common, complex traits such as hypertension and diabetes, some of these studies have highlighted differences in allelic variant frequencies between European American and African American ALI patients for novel genes which may explain, in part, the complex interplay between ethnicity, sepsis and the development of ALI. In trying to understand the basis for contemporary differences in allelic frequency, which may lead to differences in susceptibility, the potential role of positive selection for genetic variants in ancestral populations is considered.

Keywords: acute lung injury; ethnicity; genetics; sepsis

Acute lung injury (ALI) is a common and devastating illness that occurs in conjunction with sepsis and other systemic inflammatory disorders. Consistent with other multigenic disorders, the ALI phenotype has a complex etiology. There are multiple sub-phenotypes (e.g., sepsis-associated ALI, trauma-associated ALI) and a continuum of clinical severity. Unlike other complex traits, ALI cannot be defined by any particular age of onset, nor is it preferentially expressed according to sex, although data suggest that older patients who develop ALI are more likely to die than are younger patients (1).

ALI is consistent, however, with a number of other complex immunologic and inflammatory diseases, to the extent that it is characterized by ethnic disparities in terms of morbidity and mortality. In a pivotal study, Moss and Mannino (2) analyzed National Center for Health Statistics data from the Multiple-Cause Mortality Files on 38,263,780 decedents and identified 333,004 individuals who had acute respiratory distress syndrome (ARDS) between 1979 and 1996. Age-adjusted annual ARDS mortality rates were calculated and demonstrated an increase from 5.0 to 8.1 deaths per 100,000 from 1979 to 1993, respectively,

with an overall decline to 7.4 in 1996. When these data were stratified by race and sex, however, African-American men as a group had the highest ARDS mortality rates with a mean annual mortality rate of 12.8 deaths per 100,000; African-American women also had higher mortality rates (7.4) compared with their white counterparts (5.4) and women in other racial groups. Although this study was not designed to address possible confounding factors such as care of patients with ARDS, socioeconomic status, or severity of illness, it did raise the notion that there is perhaps something fundamental about African ethnicity that predisposes to ARDS. The paucity of epidemiologic literature similar to this study limits our ability to further address these findings, or to examine disparities among other minority groups.

THE ROLE OF ETHNIC DIVERSITY IN GENETIC SUSCEPTIBILITY TO COMPLEX TRAITS

As a social category, ethnicity dictates (1) the physical environments in which people grow up, which may impact their access to health care and exposure to certain risk factors; (2) the health practices people adopt (e.g., to what extent they seek professional medical care); and (3) the health care they receive (even when other socioeconomic variables are controlled). Each of these factors may arguably impact upon measures of disease comparing one subgroup of the population to another (e.g., incidence, prevalence, mortality).

The role of ethnicity in the epidemiology of complex inflammatory and immunologic diseases other than sepsis and ALI is well documented. Consider that, within the United States, there are striking disparities in disease prevalence for many of the common disorders characterized by inflammation and/or altered immunologic responses, including hypertension (3), non-insulin-dependent diabetes mellitus (NIDDM) (4), asthma (5), and obesity (for review, *see* Reference 6). Although ethnic differences in disease incidence and prevalence have traditionally been dismissed as a mix of environmental, social, cultural, or economic factors in etiology, genetic factors should never be ignored. Recent advances in our understanding of the structure of the human genome allow new approaches to be used to test for genetic factors controlling risk for even the most complex diseases, such as ALI. For example, contentions in recent years, often aimed at amending the historical misuse of race or ethnicity as a discriminatory tool, suggested that there is no relationship between self-identified ethnicity and the frequency of certain genetic markers; however, a cataloging of the human genome combined with efforts in the fields of anthropology, genetics, and medicine have demonstrated strong connections between genetic profiles and geographic and ancestral origins of individuals, thereby bridging the biological connection between race and genetics (7). There are increasing examples of the unequal distribution between ethnic groups of "high-risk" alleles in candidate genes associated with complex diseases, such as hypertension (8), myocardial infarction (9), Crohn's disease (10), asthma (11), and colon cancer (12).

A substantial number of genetic markers associated with in-

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flammatory and immunologic diseases also show large frequency differences among ethnically distinct populations. Examples of allelic variants that have been associated with either the presence of diseases associated with the inflammatory and/or infection pathways, or for which the putative susceptibility allele occurs at a greater frequency among individuals of African descent compared with non-Africans, include: (1) the 237G allele of the β chain of the high-affinity IgE receptor [FCER1B] (13); (2) the -589T allele of interleukin (IL)-4 (14); (3) the Ile50 allele of the IL-4 receptor α gene (15); (4) the P46L (c.224C>T) variant in the gene encoding member 1A of tumor necrosis factor receptor superfamily (TNFRSF1A) (16); (5) the -174 G/G genotype in the proinflammatory cytokine IL-6 gene (17); and (6) the -401A allele of RANTES (18). The CCR5 receptor (a common receptor for RANTES, macrophage inflammatory protein-1 α , and macrophage inflammatory protein-1 β) is preferentially expressed in Th1 cells, and has been identified as a co-entry factor for macrophage-tropic HIV strains. A 32-base pair (bp) deletion in this CCR5 gene was shown to protect from macrophage-tropic HIV infection in homozygous individuals (19, 20). More than 10% of Europeans are carriers for this 32-bp deletion, whereas this mutation is absent in African populations (21). Collectively, these data suggest that certain allelic variants for immunologic and inflammatory diseases can be more common in people of African descent.

CANDIDATE GENES FOR SEPSIS-ASSOCIATED ACUTE LUNG INJURY

In an era when marker-disease association studies are common, it remains challenging to implement such studies for ALI. Considerable progress has been made, however, toward understanding the pathogenesis of ALI at the molecular level by characterizing the profiles of uniquely expressed genes using both animal and human models (22–26). As a result, “priority candidates” for association studies have been identified in the absence of conventional genome-wide linkage approaches, which are impossible to perform because family studies are not feasible. Other probable pitfalls in the study of the genetics of ALI have been highlighted as obstacles in studying the genetics of severe sepsis (the common precursor of ALI), such that sufficient numbers of cases with homogeneous phenotypes are difficult to ascertain, and appropriate controls that best match the reference population have not been agreed upon (27). To this end, it is unfortunate that many of the publications to date are based on modest sample sizes and heterogeneous groups (e.g., mixed phenotypes, combined multiple ethnic groups, etc.). These shortcomings can result in both spurious correlations and, equally important, false negative associations.

Despite these obstacles, a growing list of genes has been generated from which we can begin to characterize the genetic etiology of this complex phenotype. Table 1 summarizes associations between variants in candidate genes and sepsis in the absence of ALI and a list of candidates specifically associated with ALI, illustrating the disproportion of published information for the two phenotypes. If we consider information collected to date (see also <http://geneticassociationdb.nih.gov>), it is reassuring that several sepsis candidate genes have reached the more stringent requirement of replication in independent populations, including IL-1 receptor antagonist (IL1RA [28–31]), plasminogen activator inhibitor 1 (PAI1 [32, 33]), Toll-like receptor-4 (TLR4 [34–36]), tumor necrosis factor- α (TNFA [31, 37–41]), and TNFB, or lymphotaxin α (LTA [28, 39]), rendering them plausible candidates. The common -308 variant in TNFA has also been associated with ARDS (42). Recently, the novel ALI candidate gene, pre-B-cell colony-enhancing factor (PBEF), was identified from

high-throughput expression profiling in animal (canine and murine) and human models of ALI, validated by real-time PCR and immunohistochemistry studies, and subsequently variants in the promoter region of PBEF were shown to confer a 7.7-fold higher risk of sepsis-associated ALI ($p < 0.001$) compared with both individuals with severe sepsis and healthy control subjects (23). Functional studies have further validated PBEF as a novel biomarker in ALI.

Regrettably, far less is known about sepsis and ALI candidate genes to the extent that the frequency of the “high-risk” variants may differ with race or ethnicity, because most studies have not tested for association in more than one ethnic group, or have not stratified cases and control subjects according to racial/ethnic affiliation (for example, from the 33 studies summarized in Table 1, 10 were focused exclusively on European/European-American populations, 6 included cases of different ethnicities but did not stratify in their analyses, and 8 do not address the role of ethnicity at all). A severe limitation is the dearth of reliable information on variant frequencies in nonwhite populations in the public databases (43). The reason for these limitations is primarily due to the fact that the majority of public single nucleotide polymorphisms (SNPs) were identified in European populations, but is also a result of the high level of false-positives that have been reported due to limited sampling of individuals (44).

Nevertheless, advances in the field of genetic epidemiology of sepsis and ALI are beginning to contribute to our understanding of these genetic disparities. A polymorphism in the gene encoding the IL-1 receptor antagonist (IL1RA), which has been implicated in sepsis (28–31), was significantly more frequent among black compared to white South Africans in a study of inflammatory bowel disease ($p = 0.0096$) (45). As illustrated in Table 1, the C/T variant at nucleotide 1,580 [C/T (1,580)] in codon 131 (Thr131Ile) in the gene encoding surfactant protein B (SP-B) has been shown to be associated with ARDS (46, 47). Like many of the other published studies, the sample size was considerably modest, and further stratified into idiopathic and exogenic; however, this is also one of the few association studies of ARDS/ALI that have been replicated (48, 49), and to this end the findings are likely to be relevant. Interestingly, the first group to publish on the role of the SPB variant and ARDS also highlighted a higher frequency of the C/T (1,580) variant in African Americans and Nigerians (50). Similarly, the frequency of the deletion allele (“D,” a 287-bp Alu repeat sequence in intron 16) in the angiotensin-converting enzyme (ACE) gene, which has also been associated with susceptibility and outcome in ARDS (51), has been shown to be the highest among African Americans compared to Native Americans or whites (52). Because the study by Marshall and coworkers (51) selected subjects with ARDS with mixed etiologies (27% pneumonia, 26% sepsis, and 11% trauma), one might suspect that the association observed is for a trait similar to ARDS but perhaps not ARDS *per se*. However, the study by Marshall and colleagues is perhaps one of the only studies in the literature to have compared cases to three different types of control subjects (two ICU groups [non-ARDS respiratory failure requiring mechanical ventilation and no respiratory failure without mechanical ventilation] and a very large group of normal control subjects), plus, in acknowledging the significant influence of ethnicity on the allele frequency of ACE variants, included only one ethnic group (Caucasians) in their study. This strength no doubt compensates for the heterogeneity of phenotype in the cases.

As part of the NHLBI-funded Program in Genomic Applications, scientists at Johns Hopkins (HOPGENE, <http://www.hopkins-genomics.org/>; Garcia, PI) and Physiologic Genomics (PhysGen, <http://pga.mcw.edu/>; Jacob, PI) developed an ALI

TABLE 1. SUMMARY OF CANDIDATE GENES FROM ASSOCIATION STUDIES ON SEPSIS AND ALI/ARDS

Gene	Gene Name	Sepsis	ALI/ARDS
ACE	Angiotensin 1-converting enzyme		Marshall and coworkers, 2002* (51)
CD14	Cluster of differentiation 14	Gibot and coworkers, 2002* (69)	
CMA1	Chymase 1		Marshall and coworkers, 2002* (81)
CSP12	Caspase-12	Saleh and coworkers, 2004 [†] (65)	
FCGR2A	Fc γ receptor IIa	Bredius and coworkers, 1994* (82)	
IFNG	IFN- γ	Stassen and coworkers, 2002 [‡] (83)	
IL1RA	Interleukin-1 receptor antagonist	Fang and coworkers, 1999*, Arnalich and coworkers, 2002*, Ma and coworkers, 2002 [§] , Russell and coworkers, 2002 [§] (28–31)	
IL6	Interleukin-6	Schluter and coworkers, 2002* (84)	
LBP	Lipopolysaccharide binding protein	Hubacek and coworkers, 2001 [§] (85)	
PAI1	Plasminogen activator inhibitor 1	Westendorp and coworkers, 1999*, Menges and coworkers, 2001* (32, 33)	
PBEF	Pre-B-cell colony enhancing factor		Ye and coworkers, 2004 (23)
SPB	Surfactant protein B		Max and coworkers, 1996 [†] , Lin and coworkers, 2000 [†] , Gong and coworkers, 2004 [‡] , Quasney and coworkers, 2004 [‡] (46–49)
TLR4	Toll-like receptor-4	Agnesse and coworkers, 2002 [‡] , Lorenz and coworkers, 2002*, Barber and coworkers, 2004 [‡] (34–36)	
TNFA	Tumor necrosis factor α	Stuber and coworkers, 1996 [†] , Mira and coworkers, 1999*, Waterer and coworkers, 2001 [†] , Russell and coworkers, 2002 [†] , O'Keefe and coworkers, 2002 [‡] , Walley and coworkers, 2002 [†] (31, 37–41)	Carratu et al., 2002 [†] (42)
TNFB/LTA	Lymphotoxin α	Fang and coworkers, 1999*, Waterer and coworkers, 2001 [†] (28, 39)	
TSP1	Thrombospondin-1		Maloney and coworkers, 2002* (86)
VEGF	Vascular endothelial growth factor	Medford and coworkers, 2002 [†] (87)	

Definition of abbreviations: ALI = acute lung injury; ARDS = acute respiratory distress syndrome.

* Dataset comprised of subjects of European descent only.

[†] Dataset comprised of subjects of African descent only.

[‡] Single dataset comprised of subjects of different ethnicities.

[§] Dataset comprised of subjects of Asian descent only.

[†] Ethnic makeup of dataset not described.

^{||} More than one independent dataset; datasets representing different ethnic groups.

genomic DNA repository using European-American and African-American patients with sepsis and sepsis-associated ALI in a collaborative enrollment network entitled “Consortium to Evaluate Lung Edema Genetics” (CELEG). This invaluable resource has served as the basis of another NHLBI-funded endeavor, the Johns Hopkins project on Genetic Modifiers in Ventilator-Associated Lung Injury, which is part of the Molecular Approaches to Ventilator-Associated Lung Injury Specialized Center of Clinically Oriented Research (SCCOR; P50HL073994-01). This program aims to tease out genes associated with sepsis-associated ALI (further dichotomized into survivors and nonsurvivors, e.g., mortality resulting from ALI) compared with genes associated with sepsis in the absence of ALI, and in addition endeavors to test the hypothesis that the frequency of “high-risk” alleles/haplotypes for ALI is higher in African Americans compared with European Americans (Figure 1). Our approach in prioritizing candidate genes has been based on the coupling of creative bioinformatics

approaches and extensive expression profiling in human and animal models of sepsis/ALI (24, 53, 54).

Although enrollment is ongoing, we have, to date, generated provocative data on several novel candidate genes from the European-American and African-American CELEG patients. In preliminary studies to confirm whether allele frequencies in candidate genes will be sufficiently high among cases and control subjects in both groups for further tests of association, we have observed substantial differences in minor allele frequencies of variants between African-American and European-American subjects in nearly all candidate genes examined so far (Table 2). Of particular interest is that, for one very common allele in the gene encoding myosin light chain kinase (MLCK), a multifunctional Ca^{2+} /calmodulin (CaM)-dependent kinase in endothelium that contributes to endothelial contraction and barrier dysfunction, the variant is absent in the European American samples tested so far. Other examples are presented in Table 2. It is our anticipation that, given the striking differences in frequencies observed to date, we will have the opportunity to test the hypothesis that the disparities observed in morbidity and mortality due to sepsis-associated ALI may in fact be due in part to the differences in frequencies of “risk,” or susceptibility alleles.

IS A “RISK” VARIANT IN ONE DISEASE “PROTECTIVE” IN ANOTHER?

Our understanding of natural selection is very limited, but growing as public information on SNPs and the identification of genome-wide signatures of natural selection become available. We have learned, for example, that while the majority of human genetic variation (80–90%) occurs among individuals within the same populations (55, 56), the variation across populations is a unique source for understanding the complexities of disease

Figure 1. A proposed candidate gene approach for a case-control design study on genetic modifiers in sepsis and sepsis-associated acute lung injury (ALI) to identify differences in frequencies of “risk” variants/haplotypes between two ethnic groups.

TABLE 2. ETHNIC DIFFERENCES IN ALLELIC FREQUENCIES OF ALI CANDIDATE GENES

Gene	Single Nucleotide Polymorphism	Minor Allelic Frequencies (%)	
		African American	European American
AOAH	rs2727831 (C/T)	27	43
	rs2727833 (T/A)	22	45
AQP1	T525C	54	11
	G578C	73	41
	A645G	35	6
CD14	C-159T	28	50
IL-6	rs2067074 (C/G)	60	31
MIF	G-173T	30	17
MLCK	T782C	61	0
PAI1	4G/5G	28	57
PBEF	C-1543T	7	31
TSP1	G1693A	44	11
	T-2002C	67	21

Definition of abbreviation: ALI = acute lung injury; AOAH = acylglycerol hydrolase (Acc. #P28039); AQP1 = aquaporin 1 (Acc. #NP_932766); CD14 = monocyte differentiation antigen CD14 (Acc. #NP_000582); IL-6 = interleukin 6 (Acc. #NP_000591); MIF = macrophage migration inhibitory factor (Acc. #P14174); MLCK = myosin light chain kinase (Acc. #NP_149109); PAI1 = plasminogen activator inhibitor-1 (Acc. #NP_000593); PBEF = pre-B cell enhancing factor (Acc. #A55927); TSP1 = thrombospondin 1 (Acc. #TSHUP1).

Percentages are based on African-American (n = 81) and European-American (n = 288) subjects with sepsis-associated ALI, sepsis only, and normal controls recruited into the Consortium for the Evaluation of Lung Edema Genetics (CELEG). Underscore represents minor allele variant in public databases.

disparity. Human genetic variation is the end result of diverse forces in nature, and adaptation via natural selection is the net effect of local, selective environmental forces (e.g., parasites, disease, diet, climate) resulting in altered patterns of genetic variation only in certain populations. Well documented examples of “positive” selection for genetic variants are relatively few and the best are nearly restricted to infectious diseases (e.g., resistance to malaria [57]) or nutritional adaptations (e.g., lactose tolerance [58, 59]). A closer examination of these selected variants may be useful in defining the genotype–phenotype correlation for genes controlling risk for complex diseases.

Consider, for example, the Duffy (*FY*) locus, a polymorphism that has long been recognized as one of the classic examples of a positive genetic selection for a marker that protects against malaria. The *FY* locus encodes two codominant alleles, *Fya* (*FY**A) and *Fyb* (*FY**B), that are expressed on erythrocytes, endothelial cells of postcapillary venules, and Purkinje cells of the cerebellum (reviewed in Reference 60). Duffy antigen is identical to an erythrocyte chemokine receptor, and the Duffy antigen was subsequently renamed Duffy antigen/receptor for chemokines (DARC) (61). A point mutation in the DARC promoter (T-46C) that selectively abolishes DARC expression on erythrocytes is the molecular basis for the Duffy-negative phenotype, *Fy*(a-/-), observed in the majority of African individuals (62). Absence of Duffy on erythrocytes confers an evolutionary advantage in malaria-endemic regions, because Duffy-negative erythrocytes are resistant to infection by *Plasmodium vivax* (57). In contrast to other chemokine receptors, DARC binds with high affinity to chemokines of both the CXC and CC classes (63) and is believed to function as a chemokine clearing receptor (64). Therefore, the lack of expression of DARC on RBCs may result in sustained levels of chemokines and could promote features of CC chemokine-mediated allergic inflammation. Thus, the Duffy genotype that is protective in the context of exposure to malaria potentially confers susceptibility to atopy, and consequently may contribute to the ethnic and racial disparities observed in allergic disease, including asthma. Ironically, the *FY* polymorphism is frequently included on panels of AIMs used to detect and adjust for stratification, when in fact it may be a disease marker.

In other cases, a “protective” association is less obvious, such as the recent findings by Saleh and colleagues (65), in which they identified a novel read-through CGA (Arg) codon resulting in a full-length caspase polypeptide (Csp12-L) that is associated with severe sepsis and a higher mortality due to sepsis (Table 1). This variant confers hyporesponsiveness to LPS-stimulated cytokine production and is present in approximately 20% of populations of African descent and all other primate and rodent species examined, but is absent in Europeans and Asians. The long variant of Csp12 results in the production of fewer proinflammatory cytokines and subsequently renders the host unable to manage early bacterial replication. However, Munford and Pugin (66) argue that the prevention of systemic inflammation, while leading to immunosuppression, is in fact “normal” and even critical for effective host defense in that it allows the homing of leukocytes to local invasions. The real question is, perhaps, what selective forces following the human migrations out of Africa conferred an advantage for an enhanced immune response to LPS and cytokine release.

By focusing on another candidate variant for sepsis, there is further support for the paradox described above. Given the pivotal role of CD14 in innate immunity as an LPS-binding receptor and initiator of an antimicrobial defense response (67, 68), variants in this gene have been the focus of susceptibility to sepsis. Although not yet replicated, Gibot and coworkers (69) demonstrated that a C→T promoter polymorphism at bp -260 in CD14 increased the relative risk of death and was significantly overrepresented among patients with septic shock compared with control subjects. Alternatively, a number of groups to date have demonstrated that the “sepsis risk” T allele is associated with lower serum total IgE levels and/or protection against asthma (70–75). In our own association studies on the role of the CD14(-260)C/T variant, we also observed lower total IgE and less severe asthma among two populations of African descent (K. C. Barnes and colleagues, unpublished observations), but the frequency of the T variant was nearly half that reported in populations with few or no subjects of African descent (70, 71, 73, 74, 76, 77) (Note: Among the CELEG participants, we observe a similar pattern of frequency for the CD14[-260] T variant; Table 2). Looked at another way, the wild-type C allele

(associated with higher total IgE levels) was more common among two populations of African descent compared with non-African populations, suggesting that there might be some preferred advantage to the phenotype resulting from the CC or CT genotype. One hypothesis could be some selective advantage conferred by high IgE producers in regions endemic for extracellular parasitic disease, since the production of very high concentrations of specific and nonspecific IgE in response to helminth antigen is associated with a protective response against infections (78) and because individuals with a history of atopy have been shown to be “protected” from helminthic parasites because they mount a stronger IgE-mediated response to worm antigen and demonstrate a lower intensity of parasitic infection compared with nonatopic individuals with the same exposure (79, 80).

SUMMARY

The field of genetic epidemiology has much to offer in disentangling the effect of heritable variation and its contribution to susceptibility of sepsis and sepsis-associated ALI. Just as the role of ethnicity has proved to be critical in both morbidity and mortality of common complex traits such as cardiovascular disease, diabetes, and asthma, advancements in the genetics of ALI—substantially aided by novel findings from well designed genomic studies—have demonstrated striking differences in “high-risk” alleles in an ever-expanding list of candidate genes. Taken together, we are optimistic that the studies described herein will provide novel insights into the genetic basis for predisposition to the development of this frustrating, critical illness. It is anticipated that findings derived from these and other efforts endeavoring to identify novel genetic modifiers of sepsis and sepsis-associated ALI, with a focus on vulnerable populations, will facilitate development of new therapies to limit adverse effects of mechanical ventilation on the acutely injured lung, and identify genetic markers that help direct other preventative and therapeutic strategies in sepsis patients, especially for those who are most at risk.

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