Fifty Years of Research in ARDS
Genomic Contributions and Opportunities

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Abstract

Clinical factors alone poorly explain acute respiratory distress syndrome (ARDS) risk and ARDS outcome. In the search for individual factors that may influence ARDS risk, the past 20 years have witnessed the identification of numerous genes and genetic variants that are associated with ARDS. The field of ARDS genomics has cycled from candidate gene association studies to bias-free approaches that identify new candidates, and increasing effort is made to understand the functional consequences that may underlie significant associations. More recently, methodologies of causal inference are being applied to maximize the information gained from genetic associations. Although challenges of sample size, both recognized and unrecognized phenotypic heterogeneity, and the paucity of early ARDS lung tissue limit some applications of the rapidly evolving field of genomic investigation, ongoing genetic research offers unique contributions to elucidating ARDS pathogenesis and the paradigm of precision ARDS medicine.

Keywords: acute respiratory distress syndrome; genetic association; genomic; gene expression

From its earliest description (1), the acute respiratory distress syndrome (ARDS) has been notable for the heterogeneity with which patients present (2). Precipitants as varied as battlefield trauma, pyelonephritis, and severe pneumonia might each result in life-threatening acute respiratory failure (3). At the pathologic level, ARDS presents as diffuse alveolar damage, edema, neutrophilic alveolitis, hemorrhage, hyaline membranes, pneumonia, or a combination of the above (4). Despite significant progress elucidating clinical risk factors and in codifying our ventilator approach for patients with ARDS (5, 6), a striking truth has been apparent to clinicians caring for patients with ARDS: clinical risk factors alone poorly predict which patients evolve and survive ARDS. Therefore, the question arises whether genetically determined ARDS risk may be playing a significant role.

The genomic contribution to ARDS is not immediately apparent. Unlike idiopathic pulmonary fibrosis and asthma, no family pedigrees of ARDS have been described. There is no perfect knockout model of ARDS to implicate one gene or pathway as the critical determinant of its pathogenesis (7, 8). Nonetheless, evolutionary pressures, from traumatic injury to historic host–pathogen interactions, have resulted in significant human genetic diversity with the potential to impact ARDS risk (9). Therefore, there may be great value in applying genomic tools to the study of ARDS, a complex trait that requires both a severe environmental insult and, likely, an individual predisposition. From endotype identification to determining useful intermediate variables, discovering novel biology, and causal inference, genomic applications are poised to make significant contributions. In this review, we consider the insights gained by studying genetic and transcriptomic variation in ARDS; highlight opportunities for genomic investigation to create new knowledge about ARDS risk, pathogenesis, and mortality; and offer a vision for how genomics could enrich efforts to develop ARDS precision medicine.

Promise and Pitfalls of Genomics to Untangle Complex Traits

Genomic research has led to significant advances in our understanding of human

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disease. Countless genetic association studies have been reported, identifying novel pathways relevant to a multitude of diseases and ripe for the development of targeted therapies. In cardiovascular disease, genetic linkage and genome-wide association studies (GWAS) have identified hundreds of genetic variants associated with cardiovascular diseases and traits, including variants in genes not previously hypothesized to be related to human pathology (10). For example, the identification of rare genetic variation in the gene proprotein covertase subtilisin/kexin type 9 (*PCSK9*) in two families with hypercholesterolemia provided novel biological insights into lipid metabolism (11). *PCSK9* variants subsequently were found to be associated with risk of coronary artery disease in addition to plasma levels of low-density lipoprotein (LDL) (12). Pharmacologic inhibitors of the *PCSK9* gene product lower LDL cholesterol, reduce the rate of myocardial infarction and mortality, and are now commercially available (13). Loss-of-function variants in *PCSK9* have also been implicated in altered pathogen lipid clearance via the LDL receptor and subsequent improved outcomes from septic shock, a major risk factor for ARDS (14).

Unique challenges in studying the ARDS phenotype have limited genetic studies of the syndrome performed to date. First, ARDS is a complication of a significant environmental insult, such as pneumonia, sepsis, or traumatic injury (15). The requirement for such a large environmental insult prevents the use of genetic linkage studies of family pedigrees to identify genetic influences on ARDS risk or mortality. Second, ARDS is a heterogeneous disease with multiple different pathogenic processes contributing differently in different patients depending on clinical factors as well as genetics (16–19). This heterogeneity may weaken the effect estimate if genetic variants influence some forms of ARDS but not others, making it difficult to identify potentially influential gene variants in traditional association studies. Third, ARDS lacks a simple diagnostic test and is underrecognized by clinicians, precluding the use of International Classification of Diseases ninth or tenth revision codes to identify cases for genetic studies, and adding a labor-intensive requirement for close phenotyping by trained physicians (20). Even with close review, the reliability of chest radiographs for the diagnosis of ARDS is limited (21, 22), introducing phenotype misclassification and further noise in genetic epidemiology research. Finally, cohorts of patients with ARDS and at-risk patients have not achieved the sample sizes typically required for GWAS that identify multiple risk variants, perhaps because ARDS is underrecognized and lacks a single diagnostic test.

Despite these challenges, there remains significant potential in genomic research, particularly as patient cohorts grow and novel approaches are applied to study ARDS. Genomic research may elucidate previously unknown mechanistic pathways important in the pathogenesis of ARDS by identifying unexpected genetic associations. It may allow for the identification of potentially causal biomarkers in ARDS pathogenesis or improve prognostication in patients at risk for ARDS. Last, genomic research has the potential to allow for the identification of subgroups of patients most likely to respond to directed therapies eventually underpinning personalized and precision medicine in ARDS.

Genetic Association Studies in ARDS: Many Genes, Small Effects

Some of the earliest studies to identify genetic risk factors for ARDS development or severity focused on genetic variants with potential functional significance—on the basis of associations with either protein or gene expression—and within genes with a strongly hypothesized role in lung injury. This candidate gene approach, where both the gene and the specific variant being genotyped were selected *a priori* as likely to alter ARDS risk, successfully identified numerous genetic variants that have subsequently been replicated as associating with ARDS risk or mortality (Figure 1). Notable examples include a potentially functional variant in the gene encoding angiotensin-converting enzyme (*ACE*) (23, 24) and variation in the surfactant protein B (*SFTPB*) gene (25). These early studies are remarkable in that the genetic associations were demonstrated with very small sample sizes, using ARDS case populations of only 50 to 100 subjects (25); the focus on genetic variants known to alter gene or protein expression may explain the larger effect size (26). Subsequent candidate gene association studies moved beyond known functional variants and used a tagging strategy to capture most of the common variation in a gene of interest, on the basis of the concept of linkage disequilibrium, whereby alleles at different loci are inherited together and thus display a nonrandom association. Although replication at the precise locus and for the same phenotype (ARDS risk) was inconsistent in larger and more varied populations, subsequent replication provided evidence for valid genetic associations in some instances (27–30). For other candidate gene studies, replication of the precise genetic variant proved elusive, but either the variant-tagging haplotype or another locus within the gene would demonstrate an association with ARDS in subsequent studies, whereas numerous genes have been associated with ARDS risk or mortality in only one population (Figure 1).

Must we always replicate a genetic association for it to be "true"? Although replication can seem a high hurdle to genetic researchers, replication is necessary due to the frequent observation that initial associations fail to reproduce in subsequent testing (31). Several factors may explain this lack of reproducibility: the initial studies may have been too small and thus prone to unstable risk estimates, the risk variant may be very rare, control populations may have been poorly chosen, genetic ancestry or population structure may not have been accounted for, or technical issues about the genotyping strategy may thwart replication. Furthermore, publication bias may limit dissemination of which variants fail to replicate. Thus, even "focused" genetic association studies examining only a single variant at a time should be expected to perform replication once a suitable population is identified. This expectation is then of paramount importance with the transition from single-gene analyses to multiplex or high-throughput genomic analyses, where association is being tested between a disease trait and hundreds, thousands, or millions of genetic variants, and the possibility of very small *P* values becomes statistically expected due to chance.

Spurred by the completion of the Human Genome Project, the technological progress in detecting and cataloging human DNA variation at "whole-genome"

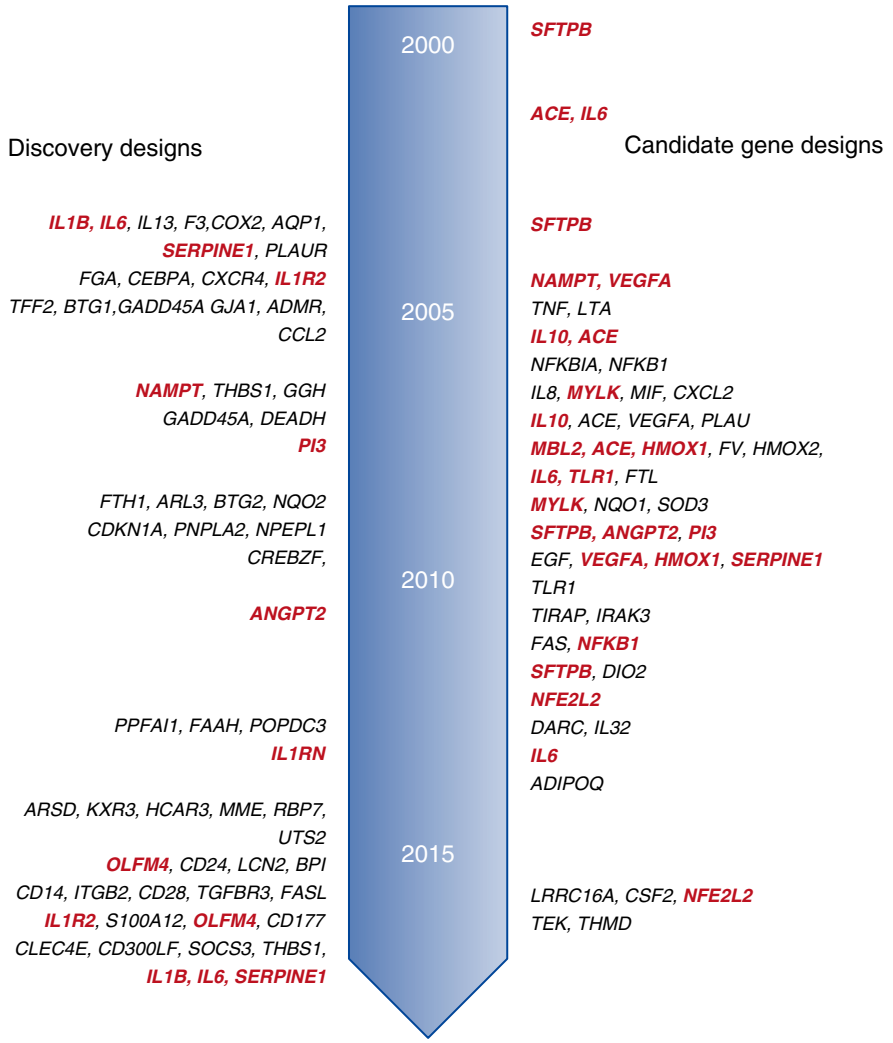

Figure 1. Selected genes that have been associated with acute respiratory distress syndrome (ARDS) risk or outcome since 2000. Although many genes were identified through a candidate gene approach (81, 86, 92–94), discovery approaches such as microarray analysis (79, 80, 82, 86, 95), whole-genome genotyping (41), and whole-exome sequencing (42) have been applied more recently. Genes are listed by their Human Genome Organisation (HUGO) gene nomenclature committee–approved symbols and are displayed at the approximate year of their publication. Genes that have replicated their ARDS association are highlighted in *red*.

scale has been nothing short of astonishing over the past 20 years (32–35). As the Human Genome Project elucidated common genetic variation across global populations, thousands and eventually millions of oligonucleotide probes were arrayed onto DNA chips (36), facilitating medium- and eventually high-throughput characterization of many single-nucleotide polymorphisms (SNPs) at once. Medium-throughput DNA arrays examined as many as 50,000 SNPs in roughly 2,000 candidate genes and identified several replicating ARDS risk genes (37–39). Furthermore, the genes implicated by these arrays often fell in pathways that might offer a pharmacologically targetable option, including angiopoietin-2 and IL-1 receptor antagonist (37, 38), because these multiplexed arrays were designed to capture high-priority candidate genes (40).

In 2012, ARDS genetics finally entered the genome-wide era with a two-stage GWAS for trauma-associated ARDS risk, leveraging a multisite collaboration and more than 800 trauma-associated ARDS cases (41). Although a landmark study for the field, this GWAS offered the sobering reality that even among a relatively homogeneous population with a single ARDS risk factor, a discovery population of 600 cases resulted in inadequate genome-wide power. Although no locus achieved genome-wide significance, more than 150 loci replicated association at marginal *P* values (0.01–0.05). One replicating SNP in the gene liprin alpha (*PPFIA1*) was also an expression quantitative trait locus (eQTL), meaning the SNP was associated with altered *PPFIA1* messenger RNA expression in stimulated B-lymphoblastic cell lines, providing evidence for the mechanism behind *PPFIA1* variation and ARDS risk (41). Moving beyond DNA array-based genotyping, an exome analysis based on next-generation sequencing of the exonic, or coding, region of the genome has also been reported for ARDS as an outgrowth of the NHLBI's Exome Sequencing Project (42). Comparing the exome of 96 patients with ARDS to 440 healthy control subjects, approximately 45,000 SNPs were investigated, and 2 SNPs were in Hardy-Weinberg equilibrium and highly associated with ARDS ($P < 3 \times 10^{-7}$). A rare intronic SNP in a putative regulatory region of the X-linked gene arylsulfatase D (*ASRD*) was present in 22% of patients with ARDS and never observed in the healthy control subjects, and a nonsynonymous coding SNP in the XK Kell blood group complex member 3 gene (*XKR3*) had a minor allele frequency of 37% in cases compared with 4% of control subjects (42). These two candidates warrant replication, and, if replicated, demonstrate the efficiency of focusing on the functional (protein-coding) or regulatory regions of the genome. Next-generation sequencing is not limited to known variants, and thus the Exome Sequencing Project data are also ripe for a rare variant analysis, which would involve counting all variants annotated to a specific gene or locus and asking whether more variation is observed in ARDS cases compared with control subjects. Such an analysis allows for "private" gene variants—variants that may have arisen *de novo* in one individual or family and thus will not be detected in another individual—to be grouped by their annotated gene (43). Although a rare variant analysis has been helpful in elucidating some potentially targetable pathways involved in complex traits like attention deficit hyperactivity disorder (44) and thus could be applied to ARDS,

the technique requires large sample sizes that may limit its application (43).

Balancing Power and Precision

Although one focus of ARDS genomic research has been to genotype or sequence increasingly larger populations in an effort to maximize statistical power, there is also a growing recognition that some of the heterogeneity of ARDS risk and outcome may be explained by different underlying biology that results in a similar clinical presentation (45). Genomic signals may be difficult to detect if all ARDS cases are analyzed together without consideration of biology, as a genetic variant that is critical to one endotype, or biologically determined subphenotype, may have no influence on a second endotype, and thus the aggregate effect may be null. Therefore, balancing the desire for ever-larger populations is a strong interest to deeply phenotype ARDS and identify potentially biologically distinct subgroups. Using clinically definable features such as ARDS-predisposing insult (sepsis vs. trauma, or pneumonia vs. nonpulmonary sepsis) has already identified unique genetic risk variants for different clinical conditions (30, 39). An approach that has been successful in asthma is to use respiratory gene expression patterns to group study subjects biologically and then further study these subgroups for differential disease mechanism or differential response to therapy (46, 47). Precision anti–IL-4 therapy for eosinophilic asthma owes credit to such studies demonstrating significant dysregulation of genes regulated by IL-4 specific to this subgroup (46, 48). When a genetic effect is very large, an extreme phenotype may result, and thus the effect may be detectable despite a very small sample size. For example, although only approximately 1% of neuroblastoma cases are considered "inherited," genome-wide screening within a small collection of affected families revealed a locus of association that was subsequently mapped to the anaplastic lymphoma kinase (*ALK*) oncogene (49), which has prompted investigation of precision anti-ALK therapy for some childhood tumors (50). How can this strategy of biological subclassification be applied to ARDS? Better profiling of whole blood, inflammatory cell, or bronchoalveolar lavage RNA, protein, and metabolite expression in ARDS may identify new subclasses that are more likely to share a common biological mechanism, which can then be investigated for underlying genomic variation. Clinicians who identify families with clustering of ARDS are encouraged to refer families to an academic ARDS center. Among clinical trial populations with ARDS, latent class analysis incorporating plasma biomarkers has identified distinct subphenotypes of ARDS that exhibit differential response to positive end-expiratory pressure and conservative fluid balance (18, 51). If these subphenotypes reflect different underlying mechanisms of injury, restricting genetic or gene expression studies to one subphenotype at a time may be more informative, by ensuring a more homogeneous case population. Furthermore, as new therapies are tested in clinical trials, prospectively collecting DNA and RNA with the intent to analyze responders and nonresponders may enable future insights.

Intermediate Traits and Causal Inference

We have highlighted that candidate gene studies tend to be limited by their failure to replicate and potentially their failure of imagination, given their requirement for hypothesizing the gene's role in ARDS (52); however, ARDS GWAS are also limited by their requirement for large sample sizes and the heterogeneity of the ARDS phenotype. One possible solution to harness the power of GWAS using large but not massive ARDS at-risk populations is to use GWAS to dissect quantitative intermediate traits with potential ARDS significance. Such traits, including physiologic variables, lab tests, or protein biomarkers, can be measured more objectively than the ARDS phenotype and are often continuous rather than dichotomous outcomes, providing more statistical power for genetic association studies. These intermediates have the potential to reduce heterogeneity and complexity, as the genetic regulation of a single plasma marker is far less complex than the regulation of ARDS, and they increase statistical power, such that populations of 200 to 400 subjects may yield significant results (53). Genetic variants that influence intermediate traits may provide mechanistic insight into ARDS and one day may identify patients most likely to benefit from ARDS therapies targeting specific mechanisms. Which intermediate traits should be genetically investigated? Potential candidates include physiologic variables such as oxygenation or lung compliance, radiologic variables such as severity of lung infiltrates, or blood biomarkers such as markers of innate immunity, endothelial or epithelial dysfunction, or coagulation.

One intermediate trait that has been evaluated in ARDS is platelet count (54, 55). Platelets are critical cells in the pathogenesis of ARDS, as they contribute to microvascular coagulation, immune activation, and endothelial damage through multiple potential pathways (56, 57). Measured in peripheral blood, decreases in platelet count may represent decreased platelet production, increased platelet destruction, and/or increased platelet activation and sequestration in sites of injury, such as the lung. The development of thrombocytopenia during critical illness is associated with poor outcomes in ARDS (58, 59) and therefore may represent an intermediate phenotype relevant to ARDS pathogenesis. Wei and colleagues tested whether genetic variation known to be associated with platelet count in ambulatory patients was also associated with platelet count among critically ill patients at risk for ARDS (60). They identified a variant in *LRRC16A*, which encodes capping protein ARP2/3 and myosin-I linker (CARMIL), a protein important in actin-based cellular processes, as associated with platelet count during critical illness. Furthermore, this variant was also associated with decreased ARDS risk partially mediated through measured platelet count. The same authors subsequently associated variation in *LRRC16A* with ARDS mortality mediated through platelets, as well as *LRRC16A* gene expression in patients with ARDS (61). *LRRC16A* had never previously been implicated in ARDS, demonstrating the power of an intermediate trait to shed light on ARDS pathogenesis. The studies demonstrating an association between the *LRRC16A* gene and platelet variables with ARDS risk and mortality are examples of mediation analysis. This approach is a formal method to test the potential mechanism by which an explanatory variable (*LRRC16A* variation) influences an

outcome (ARDS risk/mortality), and it does so by dissecting the observed association effect into a direct component and one mediated by the putative intermediate variable (in this case platelet count) (60, 61). Thus, if the mediated effect is significantly associated with the outcome, we infer that the SNP associates with outcome because the SNP quantitatively influences the intermediate variable and infer the mechanism underlying the association (62).

Another potential for genomics and intermediate traits to shed light on ARDS pathogenesis is by providing evidence to distinguish causal from noncausal biomarkers. Dozens of biomarkers have been associated with ARDS risk or mortality (63). Some of these biomarkers are likely molecules that contribute causally to ARDS, but others may be reflective of other pathogenic processes and not warrant targeting with therapeutics. Although the causality of a particular molecule in ARDS pathogenesis can only be proven in a randomized controlled trial, prioritizing which markers are likely causal from those that are not before drug development could save time and money. Drug screens to modify plasma marker expression are more efficient than to modify a complex phenotype like lung injury. Genetics has the potential to highlight potentially causal intermediates using an instrumental variable technique called Mendelian randomization analysis (MR) (64, 65). MR uses the principle that individuals are "randomized" to genetic variants during gametogenesis; therefore, genetic variation can act as an instrumental variable in the association of an intermediate trait with ARDS to estimate causality. This method is theoretically independent of confounders and immune to reverse causality bias if certain assumptions are met. In MR, genetic variants associated with an intermediate trait and ARDS are used to predict the genetic component of the intermediate trait. If the variance in the intermediate trait predicted by the genetic instrument is independently associated with outcome, then causality is suggested. Figure 2 depicts the schema of MR and causal mediation analyses.

Genetic studies using MR demonstrated the causal relationship between LDL cholesterol and coronary artery disease, supporting LDL as a therapeutic target for cardiovascular disease risk reduction and treatment (12, 66). Alternatively, large, well-powered MR studies of C-reactive protein (CRP) failed to show an association between genetically predicted CRP and coronary artery disease, suggesting increased CRP levels are indirectly related to disease pathogenesis (67, 68). In ARDS, there are many intermediate traits that may have a direct causal relationship with ARDS risk or outcomes and therefore should be considered as therapeutic targets (63). High IL-1β, a proinflammatory cytokine, is associated with poor outcomes in patients with ARDS (69). In an MR analysis using haplotypes associated with plasma IL-1β as the genetic instrument, plasma IL-1β protein concentration was suggested to causally influence 90-day mortality in sepsis (70). It is possible that IL-1β or other cytokines in the IL-1 pathway may also causally impact ARDS risk, because variation in the IL-1 pathway gene *IL1RN* has associated with ARDS risk (37).

Another potential intermediate trait of interest in ARDS is plasma angiopoietin-2 (Ang-2) concentration. Plasma Ang-2 is a biomarker of endothelial activation and permeability with a strong potential to contribute causally to ARDS pathogenesis. Ang-2 is associated with subsequent development of ARDS in those at risk and mortality in those with ARDS (71, 72). In addition, genetic association studies have implicated genetic variation in the angiopoietin-2 gene (*ANGPT2*) in ARDS risk, which was subsequently validated in a medium-throughput unbiased genetic association study in critically ill trauma patients (38, 73). For potential candidates like ang-2 with prior studies implicating both the gene and secreted protein in ARDS risk, MR methodology could provide evidence to accept or reject attempts to modify plasma ANG2 or its signaling for the prevention or treatment of ARDS. Furthermore, if identified as potentially causal toward ARDS development, plasma ANG2 might be useful for future trial enrichment, particularly if therapy targeting the ANG2 pathway were being investigated (45). The application of MR and mediation analysis to some of the many biomarkers demonstrated to associate with ARDS (74) could help propel the

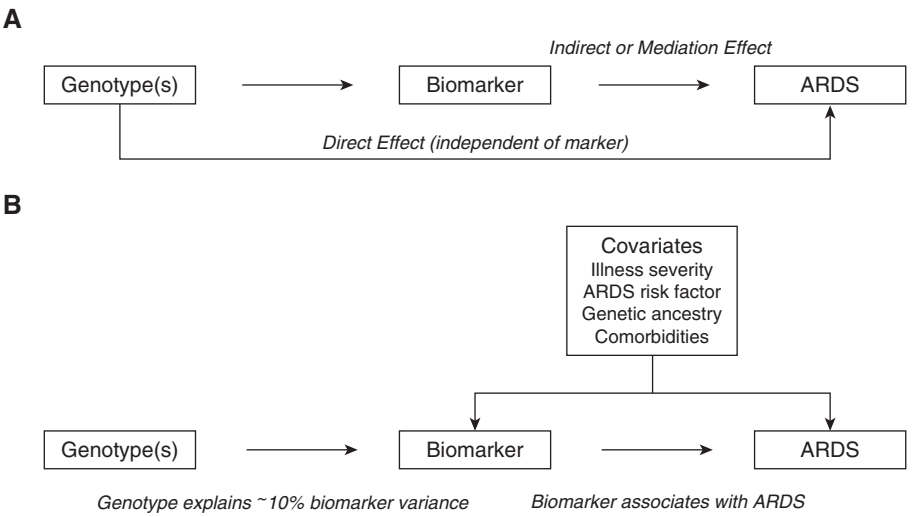

Figure 2. Genetic causal inference methodologies to understand the potential contribution of an intermediate variable on the association between genotype and outcome. (*A*) Model of a mediation analysis, in which the objective is to dissect an association into estimates of the direct and mediation effects. The mediation effect is that attributed to the intermediate marker. (*B*) Model of a Mendelian randomization analysis, which assumes that there is no direct effect between genotype and outcome and that no confounder associates with both genotype and disease. The method allows for confounders of the biomarker–disease association. Thus, if association remains significant between the genetically determined intermediate and outcome, causality is supported. Because each methodology has inherent biases, causality is best supported when multiple methodologies infer a causative role. Experimental designs are necessary to prove causation. ARDS = acute respiratory distress syndrome.

field toward precision medicine options for ARDS.

Integrative Approaches toward Systems Biology: Future Opportunities

In other complex phenotypes like asthma and lung cancer (47, 75), mRNA profiling has yielded important advances to better distinguish biologic subtypes, or endotypes, of the disease that may have differential treatment response (76, 77). One barrier to ARDS genomic research has been the limited availability of lung tissue during early ARDS due to the relative rarity of biopsy in this situation (78), and thus there is a paucity of research on human lung gene expression during ARDS. One solution to the dearth of human ARDS lung tissue is to use bioinformatic techniques to find common patterns of dysregulation among multiple animal and *in vitro* models of lung injury. In transformational work that helped to set the stage for ARDS genomic research, Simon and colleagues (79) and Grigoryev and colleagues (80) applied this approach to identify many of the candidate genes that have subsequently been replicated in human ARDS (Figure 1). Because whole blood and bronchoalveolar lavage fluid (BALf) are more accessible than lung tissue, whole-genome profiling of these tissues has also been reported in ARDS. In a cohort of patients with sepsis, neutrophil-related genes were overexpressed in the whole blood of those who subsequently developed ARDS (81). Another study implicated ferritin heavy chain as strongly up-regulated in sepsis-associated ARDS (82). Both neutrophil-related injury and iron homeostasis pathway have been implicated in ARDS pathogenesis (83–85); thus, larger whole-blood gene expression profiling of ARDS at-risk populations seems warranted. Because gene expression is cell- and location-specific, an alternative approach to whole-blood gene expression is to profile just one cell type, as was reported for alveolar macrophages isolated from BALf (86). A decoy receptor of the IL-1 pathway (*IL1R2*) and the proinflammatory gene S100 calcium binding protein A12 (*S100A12*) were enriched at both the gene and protein level in alveolar macrophages from ARDS BALf, and at the gene level in peripheral blood leukocytes, although there were significant differences between the blood leukocyte and BALf macrophage expression patterns (86). In addition, whole-genome gene expression profiling of purified neutrophils from the alveolar compartment and from circulating blood has been reported, demonstrating distinct transcriptional signatures between healthy and ARDS circulating neutrophils as well as between alveolar and peripheral blood neutrophils (87). In addition to a phenotype of delayed apoptosis and oxidative burst priming, ARDS neutrophils display markedly dysregulated glucocorticoid, IL-4, p38 mitogen-activated protein kinase, antigen presentation, and CDC52 pathway expression (87). Patients with ARDS whose neutrophils display either excessive or attenuated type I IFN signaling have significantly lower survival than patients with ARDS with mid-range IFN signaling, suggesting that host neutrophil transcriptional response may influence or prognosticate ARDS outcomes (88).

Large scale, "bias-free" approaches, such as whole-genome microarray analysis, also allow pathway analysis, which characterizes genes that are most differentially expressed between ARDS and non-ARDS by their functional, cell compartment, or signaling pathways. Pathway analysis may suggest novel processes at work in ARDS, and the pathway analyses from different experiments, such as whole-genome genotyping, microarray or RNA-sequencing, and proteomic or metabolomic screens (85, 89), could be combined in a network or "interactome" analysis to highlight critical pathways in ARDS that may converge at the DNA, RNA, and protein levels (90, 91). Although each of these discovery approaches will ultimately require controlled, hypothesis-based testing to confirm a putative role in ARDS, a systems biology approach to ARDS may prove fruitful for discovery.

Conclusions

Although relatively new to the investigation of ARDS, genomic methodologies have yielded significant insights to ARDS pathogenesis in the past 17 years. Because any one genetic variant will typically contribute only a small proportion of ARDS risk, it remains unlikely that bedside genotyping for ARDS risk variants will play a significant role in our care for patients with ARDS. In contrast, however, genomic investigation offers powerful techniques to investigate which plasma or imaging markers may play a causal role in developing ARDS or dying from ARDS, and this information then could be harnessed to improve preclinical trial efficiency, select high-risk patients for clinical trials, or even identify ARDS endotypes that may be appropriate for testing targeted therapies. Precision medicine for ARDS prevention and treatment is a realistic goal for the next 50 years in ARDS research. ■

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