Genomics in Critical Illness

20

Hector R. Wong

Abstract

The term "genomics" has morphed into an umbrella term to describe broadly the large-scale study of genes, gene products, gene variants, and their impact on health and disease. This chapter reviews the impact of genomics on critical illness and injury. The chapter will also review other "omics" such as proteomics, pharmacogenomics, epigenetics, lipidomics, and metabolomics. Gene association studies attempt to link gene variants with susceptibility to and outcomes from various forms of critical illness. Genome wide expression studies have been leveraged to elucidate novel therapeutic pathways and targets, gene expression-based subclasses of critical illness, and the discovery of candidate diagnostic and stratification biomarkers. Other "omics" disciplines are also leading to novel insights regarding the pathobiology of critical illness. For example, the discovery of neutrophil gelatinase-associated lipocalin as an early biomarker of acute kidney injury is based on trancriptomic and proteomic studies involving animals models. Comparative genomics has led to the discovery of important signaling mechanisms relevant to critical illness. For example, the discovery of Toll-like receptor 4 as the primary receptor for lipopolysaccharide is the product of comparative genomics. Finally, epigenetics is beginning to provide clues as to why recovery from critical illness may be associated for prolonged risk for subsequent critical illness. Overall, genomics-centered studies continue to evolve in the field of critical care medicine and hold the promise of substantially advancing our understanding and approach to various forms of critical illness.

Keywords

Gene expression • Proteomics • Biomarkers • Pathways • Genetics • Metabolomics • Polymorphisms • Sepsis • Acute lung injury • Genes • Transcriptomics • Microarray

Introduction

The origin of the term "genomics" is credited to T.H. Roderick of the Jackson Laboratory, Bar Harbor, Maine, during the launching of a new journal, *Genomics*, which sought to serve a "new discipline born from a marriage of molecular and cell biology with classical genetics and fostered by computational science" [1]. Since that time, for many individuals, the term "genomics" has morphed into more of an umbrella term to broadly describe the large scale study of genes, gene products, gene variants, and their impact on health and disease. This chapter will use this broader conceptual framework for reviewing the impact of genomics on critical illness and injury. The chapter will also review other "omics" such as pharmacogenomics, epigenomics, lipidomics, and metabolomics.

H.R. Wong, MD
Division of Critical Care Medicine,
Cincinnati Children's Hospital Medical Center,
University of Cincinnati College of Medicine,
3333 Burnet Avenue, Cincinnati, OH 45229, USA
e-mail: hector.wong@cchmc.org

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Gene Association Studies

It is highly plausible that much of the pathology and heterogeneity (e.g. sepsis and acute lung injury) that we encounter in the intensive care unit is substantially influenced by genetic variability. Indeed, almost 25 years ago Sorenson et al. convincingly demonstrated that premature death from infection has a stronger (although undefined) component than premature death from cardiovascular disease or cancer [2]. Despite these compelling data, however, unambiguous and well validated evidence linking specific genetic variations with critical illness have remained relatively elusive.

Most investigations attempting to link genetic variation with critical illness have focused on gene polymorphisms, defined as the regular occurrence (>1 %), in a population, of two or more alleles at a particular chromosome location. The most frequent type of polymorphism is called a single nucleotide polymorphism (SNP): a substitution, deletion, or insertion of a single nucleotide that occurs in approximately 1 per every 1,000 base pairs of human DNA. SNPs can result in an altered protein, a change in the level of normal protein expression, or no discernable change in protein function.

When SNPs cause a change in an amino acid they are said to be non-synonymous or missense SNPs (Fig. 20.1), and these are typically the type of SNPs that can lead to a change in protein function. SNPs in the promoter region of a gene or in the 3′ un-translated region can lead to changes in protein expression. Most SNPs occur in either non-coding regions or they are synonymous SNPs (i.e. variants that code for the same amino acid; Fig. 20.1) and therefore have no known direct effect on phenotype. These types of SNPs, however, may be worthy of study because although they are not causal variants they may be co-inherited along with the causal variant by a process known as linkage disequilibrium (LD), which refers to the non-random association of alleles at two or more chromosome locations, as measured by formal statistical methods. Related to the concept of LD is that of haplotype, which refers to a set of SNPs on a single chromosome that are statistically associated and typically co-inherited. These haplotype "blocks" consist of multiple linked polymorphisms and can be identified by haplotype tag SNPs. The International HapMap project is developing a haplotype map of the entire human genome as means to more effectively enable genetic association studies [3].

A classic approach to assess the impact of genetic variants on disease involves linkage analysis, which follows family members (pedigrees) for co-segregation of the disease of interest and genetic variants. This type of approach is appropriate and feasible for monogenic diseases with relatively distinct phenotypes (e.g. cystic fibrosis), but is generally not applicable to common ICU conditions such as sepsis and acute lung injury, as it is not often feasible to obtain

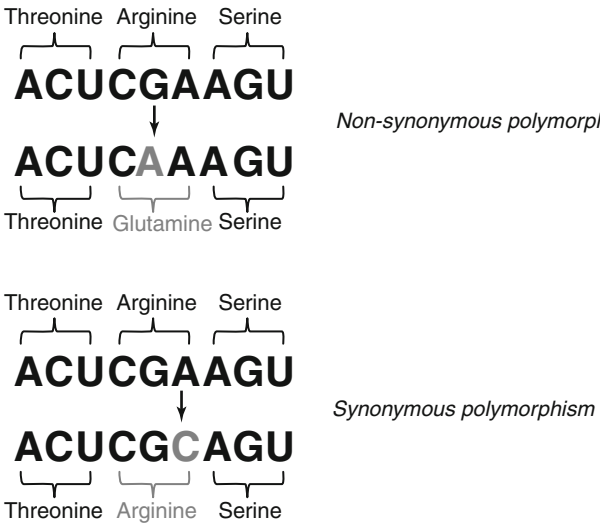

Fig. 20.1 Examples of non-synonymous and synonymous substitution polymorphisms. In the *top panel*, a change in the second amino acid for the arginine codon, from a "G" to an "A", leads to a change in the amino acid to glutamine (non-synonymous). In the *bottom panel*, a change in the third amino acid for the arginine codon, from an "A" to a "C" does not change the amino acid (synonymous)

unambiguous histories of critical illness in family members and it is not biologically plausible that sepsis or acute lung injury are monogenic syndromes. Consequently, the most common study design in the setting of critical illness is an association study, of which there are two types: case-control and cohort studies [4].

Apart from study design, another important factor in conducting genetics research in critical illness involves choosing the method for assessing genetic variation. The two primary choices are genome-wide association studies (GWAS) and candidate gene association studies. GWAS involves the simultaneous interrogation of thousands of polymorphisms. This approach is comprehensive, discovery-oriented, and relatively bias free in that the investigator makes no a priori assumptions regarding associations between any particular polymorphism and the disease of interest. This approach is relatively expensive, but cost is progressively becoming less of an issue with rapid advances in sequencing and chip technology. The main challenge that comes with GWAS is the need for a large number of patients and the application of appropriate and complex statistical methods to reduce the rate of false discovery.

Candidate gene association studies are more focused and more rooted in the traditional scientific method (i.e. hypothesis testing). In this approach the investigator focuses on a specific polymorphism, or a discrete set of polymorphisms, based on known biology, that potentially links a candidate gene to the disease of interest. This approach is less daunting from an analysis standpoint, but can be limited by investigator bias and has high potential for missing causal polymorphisms. Whichever of the two approaches one chooses, there

Table 20.1 Characteristics of an ideal genetic association study

The study should have an a priori hypothesis
Large sample size and small p values
The association between the gene and the disease of interest should have biological plausibility
The allele should affect the gene product in a physiologically meaningful way
There should be an initial study and an independent replication (validation)
The gene association should be observed in the context of both family- and population-based control cohorts
Cases should be clearly defined and should represent a spectrum of disease severity
Cases and controls should be well matched for environmental risk factors
Cases and controls should be well matched for ethnicity
Potential confounders should be presented and statistically analyzed
Allele equilibrium should be reported (Hardy-Weinberg equilibrium)
Power calculations should be targeted toward detection of a positive association

are a number of factors that impact the quality (or lack thereof) of an "ideal" gene association study. These qualities have been reviewed elsewhere [5–7] and are summarized in Table 20.1.

A large number of gene association studies have been published in the critical care literature and the reader is directed toward some recent reviews on the topic [8–12]. Table 20.2 provides a selected group of studies focused on sepsis [13–30]. Gene association studies should be conducted in the critically ill population. The heterogeneous clinical responses and presentations that we observe daily at the bedside provide the necessary general rationale that genetic polymorphisms have an important impact on our patients, in terms of disease susceptibility and disease outcome. While conducting these studies in the context of critical illness is particularly challenging, we must nonetheless seek to conduct these studies with as much rigor as that of our colleagues in other fields. One potential solution to meeting

Table 20.2 Selected gene association studies related to sepsis

Reference	Gene/polymorphism	Main findings
Lorenz et al. [22]	Toll-like receptor 4 (TLR4) polymorphisms that reduce responsiveness to endotoxin (Asp299Gly and Thr39Ile)	The TLR4 Asp299Gly allele was found exclusively in adult patients with septic shock. Patients with the TLR4 Asp299Gly/Thr399Ile alleles had a higher prevalence of gram negative infections
Agnese et al. [13]	TLR4 polymorphisms: Asp299Gly and Thr39Ile	Adult patients with these alleles had a higher incidence of gram negative infections
Multiple	TLR4 polymorphisms: Asp299Gly and Thr39Ile	Children with the Asp299Gly allele have increased risk of urinary tract infection [20], but this allele does not appear to influence susceptibility to or severity of meningococcal septic shock in children [14, 28]
Kutukculer et al. [21]	TLR2 polymorphism that reduces responsiveness to cell wall components of gram positive bacteria (Arg753Gln)	Children with recurrent infections were more frequently heterozygous for the Arg753Gln allele
Tabel et al. [29]	TLR2 polymorphism: Arg753Gln	Children with the Arg753Gln had a higher incidence of urinary tract infections
Mira et al. [25]	Tumor necrosis factor-α (TNFα) promoter polymorphism: TNF1 (guanine at -308A and TNF2 (adenosine at -308A). TNF2 allele associated with increased production of TNFα	TNF2 associated with susceptibility to septic shock and death due to septic shock
Nadel et al. [26]	TNF1 and TNF2 alleles	More deaths and increased illness severity in children with the TNF2 allele and meningococcal sepsis
McArthur et al. [23]	Lymphotoxin-α: +250A and +250G. +250A allele associated with increased TNFα production	Bacteremic children with the AA genotype had a higher mortality rate from sepsis
Read et al. [27]	Polymorphisms of interleukin-1 (IL1B (−511)) and IL-1 receptor antagonist (IL1RN(+2018))	Patients with the IL1B(−511) allele were more likely to survive meningococcal sepsis. The combination of the IL1B(−511) and the rare IL1RN(+2018) allele decreased the likelihood of surviving meningococcal sepsis
Endler et al. [16]	Multiple polymorphisms for the IL-1 locus	The IL1RA(+2018) polymorphism was associated with risk of meningococcal disease and with its outcome
Michalek et al. [24]	IL-6 polymorphisms (G-174>C and G-572>C)	Both polymorphisms could be predictors of risk of development and/or predictors of sepsis severity in children
Balding et al. [15]	Polymorphisms for IL-6, IL-1, TNFα, IL-10, and IL-1Ra	The IL-6(−174) G/G and the IL-10(−1082) A/A genotypes were more frequent among nonsurvivors of meningococcal sepsis
Multiple [17–19, 30]	Deletion/insertion (4G/5G) polymorphism of the plasminogen-activator inhibitor type-1 (PAI-1) promoter region. The 4G allele is associated with higher PAI-1 plasma levels	The 4G allele increases susceptibility to and severity of septic shock, and increased risk of mortality in children with meningococcal sepsis

this challenge is the development of multi-institutional and multi-national research consortia specifically dedicated to gene association studies.

Genome-Wide Expression Profiling

Genome-wide expression profiling (a.k.a. transcriptomics) refers to the simultaneous and efficient measurement of steady-state mRNA abundance of thousands of transcripts from a given tissue source. The general approach involves variations of microarray technology [31–33], and there is a new, potentially more powerful technique referred to as RNA Sequencing (RNA Seq) [34]. While gene expression profiling has important limitations, this discovery-oriented approach has nonetheless provided an unprecedented opportunity to gain a broader, genome-level "picture" of complex and heterogeneous clinical syndromes encountered in critical care medicine. In addition, this genome-level approach has the potential to reduce investigator bias, and thus increase discovery capability, in as much as all genes are potentially interrogated, rather than a specific set of genes chosen by the investigator based on a priori and potentially biased assumptions. Genome-wide expression profiling in sepsis will be discussed below as an example of how this approach can be applied to critically ill patients. All of the studies discussed below have used the blood compartment as the RNA source.

Several fundamental physiologic and biologic principles of the sepsis paradigms are derived from experiments involving human volunteers subjected to intravenous endotoxin challenge [35–38]. More recently, the genome-level response during experimental human endotoxemia has been studied using microarray technology [39–41]. For example, Talwar et al. compared eight volunteers challenged with intravenous endotoxin to four controls challenged with saline [39]. Mononuclear cell-specific RNA was obtained at four different time points after endotoxin challenge and analyzed via microarray. As expected, a large number of transcripts related to inflammation and innate immunity were substantially up regulated in response to endotoxin challenge. Interestingly, the peak transcriptomic response to the single endotoxin challenge occurred within six hours and mRNA levels generally returned to control levels within 24 h. The investigators also reported endotoxin-mediated differential regulation of over 100 genes not typically associated with acute inflammation.

Genome-wide expression has also been conducted in critically ill patients with sepsis and septic shock. These studies present considerable experimental challenges due to the inherent heterogeneity of clinical sepsis and septic shock. Nonetheless, several studies have provided novel insight into the overall genome-level response to sepsis [42–53]. A common theme across many of these studies is the massive up regulation of inflammation- and innate immunity-related genes in patients with sepsis and septic shock. These observations are not intrinsically novel, but they are consistent with the long-standing sepsis paradigms centered on a hyperactive inflammatory response, and thus provide a component of biological plausibility with regard to overall microarray data output in the context of clinical sepsis.

Another common paradigm in the sepsis field involves a two-phase model consisting of an initial hyper-inflammatory phase, followed by a compensatory anti-inflammatory phase, but this has been recently challenged, in large part due to the multiple failures of interventional clinical trials founded on this paradigm [54–56]. Recently, Tang et al. conducted a formal systematic review of a carefully selected group of microarray-based human sepsis studies [33]. The major conclusion of this systematic review is that, in aggregate, the transcriptome-level data does not consistently separate sepsis into distinct pro- and anti-inflammatory phases. This conclusion has been questioned [57], but is supported by several recent cytokine- and inflammatory mediator-based studies in clinical and experimental sepsis [58–60].

Another prevailing paradigm in the sepsis field involves the concept of immune-paralysis, or immune-suppression, which frames sepsis as an adaptive immune problem and the inability to adequately clear infection [61, 62]. Recently, this paradigm was elegantly corroborated in mice subjected to sepsis and rescued by administration of interleukin-7, an anti-apoptotic cytokine essential for lymphocyte survival and expansion [63, 64]. In studies focused on mononuclear cell-specific expression profiles, Tang et al. have reported early repression of adaptive immunity genes in patients with sepsis [48, 50]. Finally, multiple studies in children with septic shock have reported, and validated, early and persistent repression of adaptive immunity-related gene programs: *T cell activation*, *T cell receptor signaling*, *and antigen presentation* [42, 47, 51–53, 65–67]. Thus, the concept of adaptive immune dysfunction as an early and prominent feature of clinical sepsis and septic shock seems to be well supported by the available genome-wide expression data.

Developmental age is thought to be a major contributor to sepsis heterogeneity. Recently, a microarray-based study in children with septic shock corroborated this concept at the genomic level [68]. Four developmental age groups of children were compared based on whole-blood derived gene expression profiles. Children in the "neonate" group (<28 days of age) demonstrated a unique expression profile relative to older children. For example, children in the neonate group demonstrated widespread repression of genes corresponding to the triggering receptor expressed on myeloid cells 1 (TREM-1) pathway. TREM-1 is critical for amplification of the inflammatory response to microbial products and there has been recent interest in blockade of the TREM-1 signaling pathway in septic shock [69]. The observation that

TREM-1 signaling may not be relevant in neonates with septic shock, illustrates how some candidate therapeutic strategies for septic shock may not have biological plausibility in certain developmental age groups.

Apart from providing a broad, genome-level view of sepsis biology, as described above, genome-wide expression profiling also provides an opportunity to discover previously unrecognized, or unconsidered, targets and pathways relevant to sepsis biology. For example, using a combination of clinical expression profiling and in vitro approaches, Pathan et al. have identified interleukin-6 as a major contributor to myocardial depression in patients with meningococcal sepsis [70]. In another example, Pachot et al. identified a set of genes differentially regulated between adult survivors and non-survivors with septic shock. The gene most highly expressed in survivors, relative to non-survivors, was that of the chemokine receptor, CX3CR1 (fractalkine receptor) [44]. In a subsequent validation study, these same investigators provided further evidence supporting the novel concept that dysregulation of CX3CR1 in monocytes contributes to immune-paralysis in human sepsis [71].

A number of studies in children with septic shock have documented early and persistent repression of gene programs directly related to zinc homeostasis, in combination with low serum zinc concentrations [42, 47, 51, 53, 65]. Since normal zinc homeostasis is absolutely critical for normal immune function [72], these observations have raised the possibility of zinc supplementation as a potentially safe and low cost therapeutic strategy in clinical septic shock and other forms of critical illness [73–75]. Importantly, Knoell et al. have independently corroborated that zinc deficiency is detrimental, and that zinc supplementation is highly beneficial, in experimental sepsis [76, 77]. Additional studies by Knoell et al. have corroborated decreased plasma zinc concentrations in patients with sepsis, and that low plasma zinc concentrations correlate with higher illness severity [78]. Furthermore, plasma zinc concentrations correlate inversely with monocyte expression of the zinc transporter gene SLC39A8 (a.k.a. ZIP8) [78, 79]. Interestingly, microarray-based studies in children with septic shock have reported high level SLC39A8 expression in non-survivors, relative to survivors [53]. Despite the intriguing convergence of these data from independent laboratories, the safety and efficacy of zinc supplementation in clinical sepsis remains to be directly demonstrated and is a current area of active investigation.

In the aforementioned studies involving children with septic shock, metalloproteinase-8 (MMP-8) has consistently been the highest expressed gene in patients with septic shock, relative to normal controls [42, 47, 51–53, 65, 68]. In addition, MMP-8 is more highly expressed in patients with septic shock, compared to patients with sepsis, and in septic shock non-survivors, compared to septic shock survivors [80]. MMP-8 is also known as neutrophil collagenase because it is a neutrophil-derived protease that cleaves collagen in the extracellular matrix (ECM), but MMP-8 is also known to have other cellular sources and non-ECM substrates, including chemokines and cytokines [81]. The consistently high level expression of MMP-8 in clinical septic shock recently stimulated the formal study of MMP-8 in experimental sepsis. These studies demonstrated that either genetic ablation of MMP-8, or pharmacologic inhibition of MMP-8 activity, confers a significant survival advantage in a murine model of sepsis [80]. While these studies require further development and validation, the findings are intriguing given that there exist a number of drugs to effectively inhibit MMP-8 activity in the clinical setting [82].

Another potential application of genome-wide expression profiling is the discovery of candidate biomarkers [83]. A daily conundrum in the intensive care unit is the ability to distinguish which patients that meet criteria for systemic inflammatory response syndrome (SIRS) are infected, and which patients with SIRS are not infected. Accordingly, there are ongoing microarray-based efforts to discover diagnostic biomarkers for sepsis. Several investigators have reported genome-level signatures that can distinguish patients with SIRS (not infected) from patients with sepsis [43, 46, 50, 84]. A substantial amount of work, including validation, remains to be done in order to leverage these datasets into clinically applicable diagnostic biomarkers, but the datasets nonetheless provide a foundation for the derivation and development of diagnostic biomarkers for sepsis.

Investigators have also applied microarray technology to address other important diagnostic clinical challenges directly related to infection. Cobb et al. have reported an expression signature (the "ribonucleogram") having the potential to predict ventilator-associated pneumonia in critically ill blunt trauma patients up to 4 days before traditional clinical recognition [85, 86]. Similarly, Ramilo et al. have reported expression signatures that can distinguish Influenza A infection from bacterial infection, and *E. coli* infection from *S. aureus* infection, in hospitalized febrile children [87].

Another aspect of biomarker development in sepsis surrounds stratification (outcome) biomarkers. In theory, any gene that is consistently differentially regulated between survivors and non-survivors in a microarray dataset may warrant further investigation and validation as a potential stratification biomarker. As mentioned previously, Pachot et al., using a microarray data set, have identified CX3CR1 as a potential stratification biomarker in sepsis [44, 71]. Similarly, Nowak et al. have leveraged microarray data to identify chemokine (C-C motif) ligand 4 (CCL4) as a stratification biomarker in children with septic shock [88]. Both candidate stratification biomarkers, however, require further validation.

Interleukin-8 (IL-8) has emerged as a robust stratification biomarker in children with septic shock [89], and the rationale for pursuing IL-8 stemmed directly from

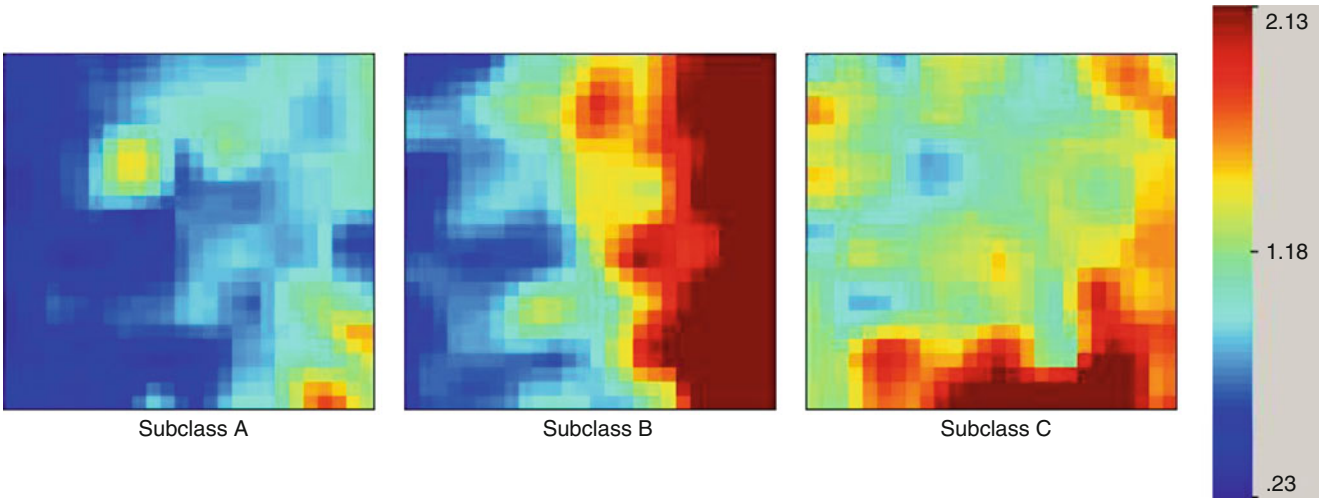

Fig. 20.2 Examples of gene expression mosaics for individual patients in septic shock subclasses A, B, and C, respectively [66, 67]. The expression mosaics represent the expression patterns of same 100-class defining genes corresponding to adaptive immunity, glucocorticoid receptor signaling, and the peroxisome proliferator-activated receptor-α signaling pathway. The *color bar* on the right depicts the relative intensity of gene expression. Patients in subclass A have a higher level of illness severity as measured by mortality, degree of organ failure, and illness severity score

microarray-based studies that identified IL-8 as one of the more highly expressed genes in pediatric non-survivors of septic shock, compared to survivors [53]. Subsequent studies in a derivation cohort of patients demonstrated that serum IL-8 protein levels, measured within 24 h of presentation to the intensive care unit with septic shock, could predict survival in pediatric septic shock with a probability of 95 % [89]. The robustness of IL-8 as a stratification biomarker was subsequently validated in a completely independent cohort of children with septic shock. Consequently, it has been proposed that IL-8 could be used in future pediatric septic shock interventional trials as a means to *exclude* patients having a high likelihood of survival with standard care, as a means of improving the risk to benefit ratio of a given intervention. This type of stratification strategy would be particularly applicable for an intervention that carries more than minimal risk. Interestingly, it appears that IL-8-based stratification may not perform in a similarly robust manner in adults with septic shock [90], thus providing another example of how developmental age contributes to septic shock heterogeneity.

Currently, there is an ongoing effort to derive and validate a multi-biomarker sepsis outcome risk model in pediatric septic shock. The foundation of this effort is the relatively unbiased selection of a panel of candidate outcome biomarkers using microarray data from a large cohort of children with septic shock [83, 91].

Viewing septic shock as a highly heterogeneous syndrome implies the existence of "disease subclasses", in an analogous manner to that encountered in the oncology field [56]. Recently, there has been an attempt to identify septic shock subclasses in children based exclusively on genome-wide expression profiling [65]. Complete microarray data from a large cohort of children with septic shock, representing the first 24 h of admission, were used to identify septic shock subclasses based exclusively on unsupervised hierarchical gene clustering. Patients with statistically similar gene expression patterns were grouped into one of three subclasses (subclasses "A", "B", or "C") and subsequently the clinical database was mined to determine if there were any phenotypic differences between the three subclasses. Patients in subclass A had a significantly higher level of illness severity as measured by mortality, organ failure, and illness severity score. In addition, the gene expression patterns that distinguished the subclasses were distilled to a 100 gene expression signature corresponding to adaptive immunity, glucocorticoid receptor signaling, and the peroxisome proliferator-activated receptor-α signaling pathway. Of note, the genes corresponding to these functional annotations were generally repressed in the subclass of patients with the higher level of illness severity (i.e. subclass A patients).

In a subsequent study, the expression patterns of the 100 subclass-defining genes were depicted using visually intuitive gene expression mosaics and shown to a panel of clinicians with no formal bioinformatic training and blinded to the actual patient subclasses (Fig. 20.2). The clinicians were able to allocate patients into the respective subclasses with a high degree of sensitivity and specificity [67]. The ability to identify a subclass of children with a higher illness severity was further corroborated when the gene expression-based subclassification strategy was applied to a separate validation cohort of children with septic shock [66]. Collectively, these studies demonstrate the feasibility of subclassifying patients with septic shock, in a clinically relevant manner, based on the expression patterns of a discrete set of genes

having relevance to sepsis biology. These features are consistent with the concept of "theranostics" in which molecular based diagnostic tools also have the potential to direct therapy [92]. The availability of clinical microfluidics [93] and digital mRNA measurement technology [94] may allow for clinical feasibility of measuring the 100 class-defining genes in a timely manner that is suitable to direct patient care or stratification for clinical trials.

Proteomics

It is well known that the degree of mRNA expression does not necessarily correlate with the degree of protein expression, and that protein function is frequently dependent on post-translational modifications. Accordingly, an important limitation of the gene expression profiling approach described above, which is focused on mRNA expression, is that it provides no direct information regarding gene end products, proteins, which ultimately carry out gene function. Accordingly, the discipline of "proteomics" has evolved to address this limitation and proteomic approaches are being increasingly applied to critical illness [93, 95–98].

As the name implies, proteomics involves the large scale analysis, including structure and function, of proteins from biological fluids and tissues. The technological armamentarium for proteomic research includes two-dimensional gel electrophoresis, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), liquid chromatography coupled to electrospray ionization-tandem MS (LC-ESI MS), surface-enhanced laser desorption/ionization coupled to TOF MS (SELDI-TOF MS), capillary electrophoresis coupled to MS, and protein microarrays [95, 96]. The broad concept of proteomics is analogous to that of transcriptomics: large scale analysis of the proteomic response during health and disease as a means of unbiased discovery.

One major application of proteomics is the discovery of candidate biomarkers [83, 99]. Human blood, a primary target for biomarker discovery and development, has been described as a highly comprehensive and readily accessible proteome potentially providing a representation of all body tissues during health and disease. However other tissues and body fluids (a.k.a. "proximal" fluids), as well as animal models, can be used for proteomics-based biomarker discovery. The discovery of neutrophil gelatinase-associated lipocalin (NGAL) as biomarker for acute kidney injury (AKI) well illustrates the discovery potential of proteomics, as well as the use of proximal fluids and animal models in the biomarker discovery phase.

NGAL is now recognized as a robust biomarker for AKI in certain populations of critically ill patients, including children [100–102]. NGAL was initially identified as a candidate AKI biomarker in rodent models of kidney ischemia [103, 104]. Analyses of the kidney parenchymal transcriptome and the urine proteome demonstrated that NGAL was one of the most abundant genes expressed in rodents subjected to experimental renal ischemia. The use of kidney tissue and urine as the biological materials was a key component of the discovery phase in that they directly represent, or are in close proximity to, the tissue of interest (i.e. the kidney), are therefore likely to be enriched for kidney-specific candidate biomarkers, and the urine proteome is several orders of magnitude less complex than the blood proteome. Thus, an unbiased approach based on biological samples from an experimental animal model enabled the discovery of a candidate diagnostic biomarker (i.e. NGAL) that may have not been readily evident using more traditional approaches.

Comparative Genomics

The ability to reliably and efficiently sequence entire genomes from a broad variety of species, including humans, has enabled the field of comparative genomics. At its most fundamental level, comparative genomics involves the analysis and comparison of genomes from different species as a means to better understand how species have evolved. Another application of comparative genomics, more directly relevant to critical care medicine, is to understand the function of human genes by examining their respective homologues in less complex organisms such as worms, flies, and mice. The identification of Toll-like receptor 4 (TLR4) as the pattern recognition receptor for lipopolysaccharide (LPS; endotoxin), and the programmed cell death process known as "apoptosis", are two relevant examples of how comparative genomics has impacted the field of critical care medicine.

TLR4 is now well known as the cellular receptor that allows cells to recognize and respond to LPS from gram negative bacteria, and several other TLRs are now known to be receptors for other classes of pathogens [105]. In addition, there is considerable interest in targeting TLR4 as a therapeutic strategy in clinical sepsis [106, 107]. The discovery of TLR4 as the LPS receptor has been comprehensively reviewed by Beutler and Poltorak [108]. Briefly, although it was known for some time that LPS was responsible for the clinical manifestations of gram negative sepsis, the cellular receptor for LPS remained unknown until data from *Drosophila* and mutant mice converged to identify TLR4 as the receptor for LPS. The Toll gene was recognized a key component of *Drosophila* immunity, and was subsequently found to have homology with the human interleukin-1 receptor. Relatively in parallel, mutant mice were discovered that were resistant to LPS, but highly susceptible to gram negative infections. Through a complex series of gene mapping experiments, the mutant locus conferring this abnormal response to LPS in mice was identified as TLR4, which was

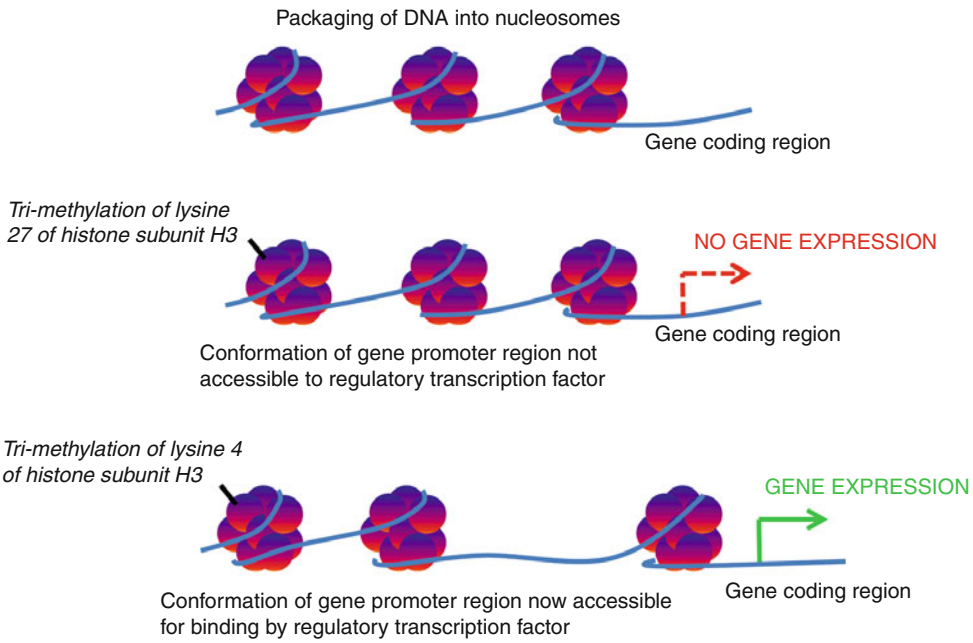

Fig. 20.3 Schematic example of epigenetic regulation of gene expression. The *upper panel* illustrates the basic packaging of DNA into nucleosomes by winding around histone cores. The *middle panel* illustrates that the addition of three methyl groups to lysine 27 of histone subunit H3 leads to a DNA confirmation that does not allow for transcription factor binding to the gene promoter region, thus repressing gene expression. The *bottom panel* illustrates that the addition of three methyl groups to lysine 4 of histone subunit H3 leads to a DNA confirmation that allows transcription factor binding to the gene promoter region, thus facilitating gene expression

found to share components with the IL-1 signaling cascade. Thereafter, with the aid of comparative genomics, the human homologue of TLR4 was identified.

The process of programmed cell death, or apoptosis, has become a focus of critical care medicine research in several areas including traumatic brain injury [109], sepsis [110], and acute lung injury [111]. The history of our understanding of apoptosis and its mechanisms has been comprehensively reviewed by several authors [112, 113]. What we know today about apoptotic mechanisms began with observations in the roundworm, *C. elegans*, which produces 1,090 somatic cells during its development, but 131 of these cells are not present in the adult due to programmed cell death. The genes responsible for this process of programmed cell death in *C. elegans* were eventually identified, and subsequently human homologues were discovered through comparative genomics.

Epigenetics

Epigenetics refers to heritable changes in gene expression patterns that are not related to direct changes to the DNA sequence of a given gene [114]. The epigenetic mechanisms that regulate gene expression include chemical modifications of DNA (typically methylation), post-translational modifications of histones (typically acetylation, methylation, and/or phosphorylation), and micro-RNAs that regulate gene expression by binding specific mRNA molecules and targeting them for degradation. A key concept of epigenetic-mediated gene regulation is that the epigenetic modifications can be "inherited" (i.e. passed on to daughter cells) and can therefore lead to long lasting effects on gene expression.

A simplified example of epigenetic regulation of gene expression is provided in Fig. 20.3. Nucleosomes are the basic unit of DNA packaging into chromatin and chromosomes. A nucleosome consists of DNA segments wound around an octamer of histone proteins (two copies each of histones H2A, H2B, H3, and H4). The histone proteins can be modified by the addition (or removal) of methyl or acetyl groups to specific amino acids. These histone modifications can, in turn, alter DNA conformation and consequently alter the ability of transcription factors to bind DNA promoter regions. In the example provided in Fig. 20.3, the addition of three methyl groups to lysine 27 of histone subunit H3 leads to a DNA confirmation that does not allow transcription factor binding to the gene promoter, thus rendering the gene as being "off". Alternatively, the addition of three methyl groups to lysine 4 of histone subunit H3 leads to a DNA confirmation that allows transcription factor binding to the gene promoter, thus rendering the gene as being "on".

Of direct relevance to critical care medicine is the evolving concept that immunity- and inflammation-related genes are subject to epigenetic regulation [115]. For example, the phenomenon of endotoxin tolerance, whereby repeated exposure to endotoxin blunts subsequent cellular inflammatory responses, is mediated, in part by epigenetic mechanisms involving histone, chromatin, and DNA modifications [116–119]. In addition, production of some cytokines and chemokines by immune cells challenged with endotoxin appears to be partially dependent on epigenetic mechanisms [120, 121]. From a potential therapeutic standpoint, a recent study demonstrated that the administration of a compound that mimics acetylated histones disrupts chromatin complexes related to inflammatory responses in macrophages and confers protection in rodent models of sepsis [122].

As discussed in previous sections, an evolving paradigm in the sepsis field surrounds the concept of altered adaptive immunity and immune-suppression. Additionally, it is now well established that patients that recover from various forms of critical illness, sepsis in particular, are at increased risk of death for several years after discharge from the intensive care unit [123–125]. Evolving experimental data indicates that sepsis induces epigenetic changes in dendritic cells and lymphocytes that render the host immune deficient for a remarkably long period of time after the initial sepsis challenge [126–128]. Of note, one of the aforementioned genome-wide expression studies in children with septic shock reported the differential expression of a group of genes corresponding to gene networks involved in transcriptional repression and epigenetic regulation, in parallel with suppression of adaptive immunity genes [52]. Thus, it possible that our future approach to the recovering critically ill patient will need to take into consideration the epigenetic impact of critical illness.

Pharmacogenomics

The discipline of pharmacogenomics encompasses a blend of pharmacology, genomic data, and genomic technology [129]. There are two broad goals or applications of pharmacogenomics: understanding variations in drug metabolism and efficacy, and discovery of new pharmacologic targets.

Variability in patient responses to drugs is a very well-known clinical phenomenon in the intensive care unit, and much of this variability is based on genetic variation in key enzymes involved in drug metabolism [130]. The cytochrome P450 (CYP) system is responsible for liver metabolism of many drugs relevant to critical care medicine, and the isoenzymes that make up the P450 system are highly polymorphic. For example, a specific SNP of CYP3A can significantly reduce the metabolism of midazolam and tacrolimus [131, 132]. Another source of variability in patient responses to drugs is based on genetic variation of drug receptors. For example, the genes encoding for adrenoreceptors (α and β) have well described polymorphisms that alter response to various cardiovascular drugs used in the intensive care unit and can also impact survival in patients with heart failure [133]. Of particular relevance to pediatric critical care medicine, polymorphisms of the β2 adrenergic receptor are linked to altered responses to bronchodilators in patients with asthma [134].

The concept of "personalized medicine" is, in large part, centered on the knowledge obtained from the discipline of pharmacogenomics. However, while the goal of personalized medicine in the field of critical care is laudable, it has yet to be realized at the bedside of critically ill patients. One practical barrier is that a great deal of pharmacogenomic data potentially relevant to the critically ill patient is generated from healthy volunteers, rather than in the critical care setting and all of the attendant confounding factors such as shock, end organ failure, and poly-pharmacy. Nonetheless, technological advances have made it feasible to obtain pharmacogenomic data in critically ill patients in a clinically relevant time frame, thus bringing the concept of personalized medicine closer to the intensive care unit. The challenge going forward will be to conduct pharmacogenomics-based research in the critical care setting, with an emphasis on drugs with narrow therapeutic and toxic ranges, and that are substantially affected by genetic variation.

Other Branches of "Omics"

The widespread enthusiasm surrounding genomic medicine, coupled with rapidly advancing technologies, have fostered the development of other forms of "omic" disciplines centered on discovery via high throughput generation of large data sets. Metabolomics involves the large scale analysis of endogenous metabolites (e.g. amino acids, carbohydrates, lactate, acetate, etc.) in blood, urine, and other biological specimens. This approach is potentially highly complementary to transcriptomics and proteomics in that it provides information about the end products of gene function, and is now beginning to generate interest in the field of critical care medicine [135]. Lipidomics is conceptually related to metabolomics, but as the name implies, it is focused on large scale analysis of lipid metabolism within a biological system [136]. Degradomics focuses on large scale analysis and discovery of protease substrates [137]. The Human Microbiome Project was launched in 2008 to develop a comprehensive catalogue of the entire community of microorganisms that reside in five anatomical locations: oral, skin, vagina, gut, and respiratory tract. The project includes genome sequencing of the identified organisms, and has the ultimate goal of elucidating how the human microbiome contributes to health and disease. Finally, there is the demanding concept of the "interactome" which seeks to combine and integrate knowledge from the various "omics" fields under the umbrella of systems biology [138, 139].

Conclusion

Despite the widespread optimism surrounding the completion of the human genome project, the promise of genomic medicine has yet to be realized at the bedside of critically ill patients. The emerging data nonetheless provide hope that ongoing advances in genomic science will eventually lead to meaningful advances in our collective approach to critical illness. Realizing this goal will require substantial resources, thoughtful prioritization, multi-center collaborations, and interactions between diverse disciplines including genetics, complex statistics, computer science, molecular biology, physics, engineering, industry, and of course, the clinicians who provide critical care.

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