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Genetic Polymorphisms in Critical Care and Illness

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Introduction

Although the vast majority of nuclear DNA is identical from one person to the next, there is a small fraction of DNA sequence (~0.1%) that varies among individuals. The variations in DNA sequence found within regions that make up genes are responsible for the genetically determined variation in our physical characteristics, our physiology, and our personality traits. Genetic variability also appears to be involved in susceptibility to some diseases, as well as therapeutic responses to treatment. Recent data have also suggested that genetic variations may affect the severity of some illnesses, thereby impacting the final outcome of these illnesses. In this chapter, we explore the evidence for whether genetic variation has an impact on critical illness and response to injury. We discuss how genetic variations may influence susceptibility to, severity of, and outcome from critical illness and injury and how they may help to identify risk factors for complications in children in the pediatric intensive care unit (PICU).

Genetic Polymorphisms

The sequencing of the human genome has revealed that many genes are polymorphic, that is, there are small differences in DNA sequences among individuals. Polymorphic genes are genes in which variation at a specific site is found in greater than 1% of the general population. The sites that are variable within the genes are referred to as *polymorphic sites*. The polymorphisms in DNA sequences may exist in several forms, with the most frequent form being a single nucleotide polymorphism (SNP) caused by a base pair substitution. In addition, polymorphisms within genes may also be caused by insertions or deletions of fragments of DNA or to the presence of a variable number of tandem repeats (VNTR) of short, repetitive DNA sequences.

Polymorphic sites can exist in coding and noncoding regions of the gene. They can have no effect, or they can influence the activity and/or level of the resulting protein, thereby affecting cell function. When present in the coding sequences of the gene, these variations can result in an alteration in the amino acid sequence of the protein that can affect the structure and function of the protein. When the polymorphic site exists in a noncoding region of the gene, it can affect the regulation of gene transcription, resulting in altered levels of protein product in the cell.

Genotyping of Polymorphic Sites

Although biochemical analyses of proteins have indicated that protein products are polymorphic, the first demonstration of the extent of polymorphism in the human genome was demonstrated using restriction enzymes that recognize and cut DNA at specific nucleotide sequences. Analyses of the DNA fragments generated by the action of a specific restriction enzyme on human DNA demonstrated that the size of the cleavage products differed among individuals. These restriction fragment length polymorphisms (RFLPs) are generally caused by an SNP within restriction enzyme recognition sites. After the realization that many SNPs were present in the human genome, a number of other methods were used to identify SNPs within genes [1,2].

Once a polymorphic site within a gene is identified, there are a number of methods that can be used to determine the genotype of individuals at that polymorphic site. As individuals have two copies of each gene, at any given polymorphic site an individual can be homozygous for one or the other polymorphism found at that site; or the individual may be heterozygous. Almost all genotyping techniques require amplification of the fragment of DNA containing the site of interest by the polymerase chain reaction (PCR) technique. This technique allows for the amplification of a specific region of the genome (in this case a region containing the polymorphic site) using small fragments of DNA that flank the polymorphic site as primers for the PCR. For insertions or deletions and most VNTRs, the genotype can be determined by examining the size of the PCR products by gel electrophoresis. In the case of SNPs, there are a

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number of different techniques that have been used for genotyping. Until recently most of these techniques were labor intensive, required experienced personnel, and were not conducive to genotyping many SNPs rapidly. More recently, with the increased interest in SNPs as tools for mapping genes and for candidate gene association studies, techniques for high-throughput SNP genotyping have begun to be developed. As the underlying strategies for the newer high-throughput techniques and the older, more labor intensive techniques are both based first on a reaction that discriminates which nucleotide is present at the polymorphic site and second on a technique that allows the identification of the product of the reaction, we discuss in detail several of the older techniques that are found in much of the literature published thus far to illustrate the general concepts. A brief discussion of high-throughput techniques is included at the end of this section.

Generally when genotyping an SNP, the two possible nucleotides found at the site are known from sequencing, and a technique is used to distinguish one nucleotide from the other. When the polymorphic site is within a recognition site for a restriction enzyme, the ability of the restriction enzyme to cleave the PCR product can be used to determine which nucleotide is present at the polymorphic site (Figure 16.1A). Whether the PCR product is cleaved is demonstrated by the size of the DNA as determined by electrophoresis.

Another way to determine whether a specific nucleotide is present at a polymorphic site is by performing allele-specific PCR (copies of DNA with different nucleotides at a specific polymorphic site are considered to be different alleles of the gene; Figure 16.1B). Allele-specific primers that are identical except for the last nucleotide are used in the PCR reaction. Polymerase chain reactions generate new pieces of DNA by the addition of nucleotides to the 3′ end of the primer that has hybridized to the DNA of interest, which acts as a template. If there is no match at the 3′ end of the primer, the polymerase extends the primer at a 100- to 10,000-fold lower efficiency, and no PCR product is detected. If the last nucleotide of the primer hybridizes to the specific allele it is designed to detect, a PCR product is formed if the individual has a copy of that allele. Presence or absence of the PCR product is determined by electrophoresis. To genotype an individual using the allele-specific PCR technique, two different PCR reactions are performed with one or the other allele-specific primer and a second primer common to both reactions.

Another technique that is often used to genotype SNPs is based on hybridization with allele-specific oligonucleotide (ASO) probes that are labeled so they can be detected (Figure 16.1C). Such probes differ only by a single nucleotide (the polymorphic site, which is generally in the middle of the ASO). In the simplest of these types of assays, hybridization conditions are chosen such that each ASO hybridizes only to its specific allele. The presence of one mismatched nucleotide is enough to prevent annealing under the hybridization conditions used. The DNA sequence surrounding the polymorphic site determines whether conditions can be identified in which the ASO probe hybridizes only to its matching allele and not to the other allele. If this is not possible, the less stable DNA duplex (that containing the mismatched nucleotide) can be distinguished from the perfect match by its melting temperature (Tm), which is an indicator of the stability of the duplex. The mismatched duplex is less stable and consequently has a lower Tm. This technique, however, is more complicated and time consuming.

In the past several years, new high-throughput techniques have been developed for SNP genotyping, some of which are beginning to be used in studies of critically ill patients [3,4]. These techniques include some that are performed in solution and others that are solid-phase reactions performed on supports such as beads or microarray chips. Most of these techniques use hybridization, single base pair extension or "mini-sequencing," or allele-specific PCR to distinguish one allele from another. Some of the detection techniques used include fluorescence, fluorescence polarization, and mass spectrometry. The different techniques available and their advantages and disadvantages have been reviewed by others elsewhere [1,2,5–8]. Which of these techniques will prove the most reliable and cost effective is not yet known. Certainly care will have to be used in applying these techniques, and appropriate controls will be required to illustrate reproducibility and reliability.

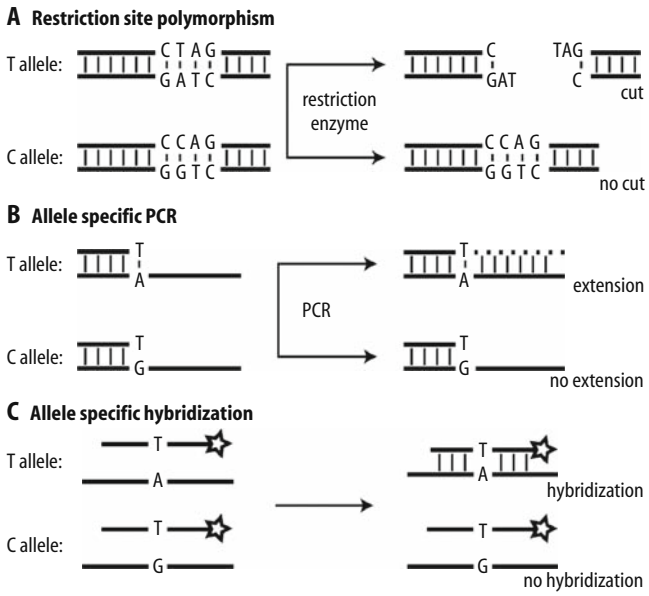

Figure 16.1. Genotyping of polymorphic sites. (A) Genotyping by restriction site polymorphism. The polymorphic site has either a T or a C. When the T is present, a restriction enzyme recognition site is formed that is cleaved in the presence of the restriction enzyme as determined by gel electrophoresis. If C is present at the site, the restriction enzyme does not cut. **(B)** Genotyping by allele-specific polymerase chain reaction (PCR). Reactions contain a common primer and an allele-specific primer ending with one of the nucleotides found at the polymorphic site. In this example, the allele-specific primer shown is for the T allele and only extension from the allele-specific primer, not the common primer. If the patient has the T allele, the last nucleotide will hybridize and extension will occur, allowing a productive PCR reaction. If the patient contains the C allele, the last nucleotide of the primer cannot hybridize and extension does not occur, resulting in no PCR product. With this technique two PCR reactions have to be performed with each patient sample. Each reaction contains a common primer and one of the two allele-specific primers. The presence of a PCR product is determined by gel electrophoresis. **(C)** Genotyping by allele-specific hybridization. Two allele-specific oligonucleotide (ASO) probes are made that are identical except for the polymorphic site. The probes are tagged for visualization as indicated by the stars. Only the allele-specific probe for the T allele is shown. The ASO will hybridize only to the DNA that contains a perfectly matched complementary sequence, in this case the T allele. Although only one reaction is shown (that containing the ASO with the T), two reactions containing the two different ASOs are performed for each patient sample. When unhybridized probe is washed away the hybridization can be visualized in a variety of ways.

Genetic Polymorphisms and Sepsis

Individuals respond to infections and antimicrobial therapies in a highly variable fashion. Most patients will recover and do well, while a small but significant portion will develop severe sepsis and

may develop multiple organ system failure, refractory hypotension, and die. This variability in the susceptibility to and outcome from sepsis, which is considered to be the most common cause of death in children in the world, has been attributed to a number of factors. These include the virulence of the etiologic agent and the length of time between onset of symptoms and initiation of treatment. However, the genetic makeup of the host also appears to play an important role in the susceptibility to and the development of sepsis, as well as its severity and outcome. For example, familial studies in which there were deaths due to severe infections demonstrated a strong genetic influence [9].

The body's inflammatory response to bacterial infection first requires recognition of pathogen-associated bacterial products. The initial recognition and the resultant response require dozens of cellular proteins, many of which are polymorphic. Genetic variation within these polymorphic genes may influence the overall response to the infection. In this section we discuss the evidence that genetic variability in specific genes plays a role in development of sepsis and its outcome [for review, see 10].

Recognition

Thus far, studies demonstrating associations between genetic polymorphisms in some of the genes coding for proteins involved in recognition and response to bacterial infection, and susceptibility to and outcome from sepsis, have implicated several genes involved with pathogen recognition as possibly being involved in the variability observed in individuals. Such genes include the toll-like receptor 4 (TLR4) gene, the mannose binding lectin (MBL) gene, and the Fcγ receptor (FcγR) genes (Table 16.1).

Lipopolysaccharide (LPS), one of the major components of the cell wall of Gram-negative bacteria, binds to a cell surface receptor composed of at least three proteins: TLR4, CD14, and MD-2 [11–15]. A number of studies suggest that variations in the TLR4 gene can generate variability in susceptibility and/or response to infection. In mice, TLR4 is required for response to LPS [16], and a single amino acid change can significantly reduce response to LPS [14,17] and enhance susceptibility to infection. In the human TLR4 gene, two SNPs have been identified that result in the replacement of an aspartic acid at amino acid position 299 with glycine and a threonine at amino acid position 399 with an isoleucine. The Gly299Ile399 variant appears to be expressed at lower levels in human airway epithelia [18], and a number of studies have demonstrated association of this variant with a reduced response to LPS as determined by examining airway reactivity or systemic cytokine response to inhaled LPS [18–20]. This variant is also associated with a diminished response to LPS in a transfected cell system using primary human epithelial cells [18]. An association of the TLR4 Gly299Ile399 variant with Gram-negative bacterial infections and septic shock [21,22] and mortality in systemic inflammatory response syndrome [23] has also been demonstrated in humans. However, the Gly299Ile399 variant showed no association with susceptibility to, or severity of, meningococcal disease [24], although other rare TLR4 mutations have been implicated in meningococcal susceptibility [25]. The lack of any association of the Gly299Ile399 variant with meningococcal disease may be explained by the observation that *Neisseria meningitides* is capable of eliciting an inflammatory response via the TLR2 receptor in the absence of LPS [26,27].

Another component of the host immune system involved in recognition of bacterial invasion is the group of leukocyte Fcγ receptors (see Table 16.1). These receptors bind to the constant region of IgG and are primarily responsible for the phagocytosis of immunoglobulin G (IgG)–coated bacteria and induction of the inflammatory response [28,29]. The human Fcγ receptors are grouped into three classes, which vary in their affinity for the various IgG subclasses. The FcγRI class consists of the FcγRIa receptor; the FcγRII class consists of FcγRIIa, FcγRIIb, and FcγRIIc; and the FcγRIII class consists of FcγRIIIa and FcγRIIIb. Genetic polymorphisms affecting function have been described in three of the Fcγ receptors [29]. The FcγRIIIa has a polymorphism at amino acid 158 resulting in either a valine (V) or phenylalanine (F) at this position, which in turn affects its affinity for IgG_1, IgG_3, and IgG_4 [30,31]. The FcγRIIIb has a polymorphism that is a four amino acid substitution (allotypes FcγRIIIb-NA1 or -NA2), resulting in differences in glycosylation [32]. This substitution alters the opsonization efficiency

Table 16.1. Genetic polymorphisms and risk of infection and sepsis.

Gene	Polymorphism*	Consequence of polymorphism
TLR4	Asp299Gly/Thr399Ile	Gly/Ile associated with decreased expression; associated with increased risk of sepsis and mortality
FCγRIIa	H131R	R associated with decreased affinity to IgG_2 and opsonization; associated with increased risk of infection and septic shock
MBL	Variants B, C, D	Variants associated with decreased levels and activity; associated with increased risk of infection
TNF-α	−308 G/A, others	A associated with increased levels; associated with increased mortality in sepsis and meningococcal disease
LT-α or TNF-β	LT-α+250 G/A	A associated with increased levels; associated with increased mortality in sepsis and bacteremia
IL-1RA	Variable 86-bp repeat	A2 associated with increased levels of IL-1RA; variable results of association studies examining risk of sepsis and mortality
IL-6	−174 G/C	G associated with increased IL-6 levels in patients, but C associated with increased levels in monocytes from neonates; associated with sepsis in neonates but not adults
IL-10	−1082 G/A, −819 C/T, −592 C/A	GCC haplotype associated with increased levels; associated with sepsis but not mortality
HSP70-2	+1267 G/A	G associated with lower mRNA levels; A associated with septic shock in adults with CAP
ACE	I/D	DD associated with increased serum and tissue levels; associated with more severe meningococcal disease
PAI-1	4G/5G	4G associated with increased levels; associated with septic shock in meningococcal disease

*The terms used for the various polymorphisms are the ones most commonly used in the literature and may refer to the nucleotide position, amino acid position, or name of the allele. This table is representative of polymorphisms examined in sepsis but does not include all such polymorphisms.

Note: TLR, toll-like receptor; Ig, immunoglobulin; MBL, mannose binding lectin; TNF, tumor necrosis factor; LT, lymphotoxin; IL-1RA, interleukin-1 receptor antagonist (GCC haplotype of the IL-10 promoter is defined by three single-site polymorphisms at −1082, −819, and −592); HSP, heat shock protein; CAP, community-acquired pneumonia; ACE, angiotensin-converting enzyme; PAI, plasminogen activator inhibitor.

required for phagocytosis of IgG_1- and IgG_3-opsonized particles [33,34]. Individuals homozygous for the FcγRIIIb-NA1 allotype appear to have more efficient phagocytosis. The FcγRIIa gene has a polymorphic site at amino acid position 131 [35,36] that results in either a histidine (FcγRIIa-H131) or an arginine (FcγRIIa-R131) at amino acid position 131. This amino acid is in the extracellular domain of the receptor, and the FcγRIIa-R131 allotype binds the Fc portion of IgG_2 with lower affinity than the more common FcγRIIa-H131 allotype [36]. In vitro studies have demonstrated reduced phagocytosis of IgG_2-opsonized particles in cells from individuals homozygous for FcγRIIa-R131 compared with cells from individuals homozygous for FcγRIIa-H131 [37,38]. Immunoglobulin G_2 is the main antibody subtype directed against encapsulated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *N. meningitides* and plays an important role in their phagocytosis [36,39,40]. Studies have examined the association between the presence of the FcγRIIa-R131 and/or the FcγRIIIb-NA2 polymorphisms in individuals and an increased susceptibility to infections, particularly meningococcal disease. Although the vast majority of reports have shown an association between infection and/or sepsis and the FcγRIIa and FcγRIIIb polymorphisms, there are two reports where no association was seen [41,42]. However, in most studies higher frequencies of the FcγRIIa-R131/R131 or FcγRIIIb-Na2/Na2 genotypes have been found in patients with meningococcal disease [43–48], particularly in patients with severe meningococcal disease [45,46] or fulminant meningococcal septic shock [43,44] when compared with a healthy control population. An association between the FcγRIIa polymorphism and infection with other encapsulated bacteria has also been reported [49,50]. Thus, genetic variation in the gene coding for at least two of the Fcγ receptors appears to influence the susceptibility to and outcome from infection with encapsulated bacteria.

Mannose binding lectin is also involved with the opsonization [51] of bacteria and binds to bacterial surface oligosaccharides N-acetyl glucosamine and mannose [52]. The heterotrimeric MBL protein contains a carbohydrate binding domain and a helical tail domain that is important in polymerization of the three peptides [53]. Polymerization of the heterotrimer is crucial for the stability of MBL. Three genetic polymorphisms have been described in MBL in the amino acids at the positions 52, 54, and 57 (referred to as variants D, C, and B, respectively). These polymorphic sites result in amino acid changes that diminish the ability of the helical tails to polymerize, resulting in an increased degradation of MBL [51,54,55] and reduced serum levels of MBL [55]. Studies have demonstrated associations among these MBL genetic polymorphisms and increased susceptibility to infections [56], hospitalizations because of infections in children [57], number of acute respiratory infections in children [58], increased risk for meningococcal infections [59], susceptibility to infections in patients with systemic lupus erythematosus [60], increased risk for recurrent respiratory infections [61], and increased susceptibility to invasive pneumococcal disease even in individuals with at least one copy of the variant polymorphism [62]. Thus, as with the genetic polymorphisms in the genes coding for the FcγRIIa and FcγRIIIb receptors, there appears to be an association between the MBL genetic variants and susceptibility to bacterial infections.

Response

Proinflammatory cytokines, such as tumor necrosis factor- α (TNF-α), interleukin-1 (IL-1), and IL-6, are produced and secreted within minutes of a pathogenic stimulus and result in the secretion of many other cytokines and chemokines. This is balanced by the subsequent release of antiinflammatory cytokines such as IL-10 and a return to baseline of cytokines and chemokines [63,64]. It is now generally accepted that an overexaggerated proinflammatory response resulting in an imbalance between the proinflammatory and antiinflammatory cytokines results in the clinical manifestation of severe sepsis and septic shock. The mechanism by which this imbalance occurs leading to exaggerated response is an area of intense research. Genetic variability within genes coding for the proinflammatory and antiinflammatory cytokines might influence this balance and could potentially influence the overall susceptibility to and outcome from the sepsis.

Tumor necrosis factor-α and the genetic polymorphisms within the regulatory regions of the gene coding for TNF-α are perhaps the most extensively studied of all sepsis-induced cytokines. We will discuss the genetic polymorphisms found in the TNF-α locus in more detail here and briefly mention other polymorphisms and association studies. As a proinflammatory cytokine, TNF-α plays a key role in the pathogenesis of the acute inflammatory response and is responsible for the activation of the inflammatory response. Tumor necrosis factor-α is also responsible for the development of the harmful effects of the systemic inflammatory response such as capillary leak, hypotension, acute respiratory distress syndrome (ARDS), and multiple organ system failure [65–69]. Several SNPs within the regulatory region of the gene coding for TNF-α have been identified that impact TNF-α production [70–77]. The most studied are the G to A transitions 308 and 238 base pairs upstream from the transcriptional start site for the TNF-α gene. In vitro studies have demonstrated that the rarer TNF-α–308A allele is associated with increased transcription [77] and increased secretion of TNF-α from LPS-stimulated macrophages [72] compared with the more common TNF-α–308G allele. In contrast, the more common TNF-α–238G allele is associated with higher TNF-α production in vitro compared with the rarer TNF-α–238A allele [78]. These polymorphisms lie near putative DNA binding sites for several transcription factors, and in vitro studies have demonstrated differential binding of nuclear proteins to DNA fragments containing either an A or a G at the TNF-α–308 position [79].

Another polymorphism associated with higher levels of TNF-α is approximately 250 base pairs downstream from the transcriptional start site for the gene coding for lymphotoxin-α (LT-α, also known as TNF-β). This site (also referred to as the TNFB allele, LT-α+250, and TNF-β+252 site; for this chapter, LT-α+250 site will be used) is approximately 3.2 kb upstream from the TNF-α gene. Higher serum levels of TNF-α have been demonstrated in septic patients with the LT-α+250A allele [73–75]. Consequently, either this region acts as an enhancer for the TNF-α gene, or it is linked to a regulatory region further downstream. In any case, the data from studies of the TNF-α–308, TNF-α–238, and LT-α+250 alleles provide convincing evidence that genetic variation within regulatory regions of the gene coding for TNF-α influences the amount of TNF-α produced.

Most association studies have suggested that the TNF-α polymorphisms influence the clinical presentation and/or outcome in children with meningococcal infections [80] or bacteremia [73] and in adults with septic shock [70,75,81,82], or community-acquired pneumonia [83]. Specifically, the frequency of the TNF-α–308A allele is higher in adults who died with septic shock [82] and in children who died from meningococcal disease than in controls [80]. Even those children who were heterozygous at this position

(TNF-α–308 G/A) were at increased risk for more fulminant meningococcal disease and death compared with those children who were homozygous for the wild-type genotype (TNF-α–308 G/G). At the LT-α+250 site, analysis of a cohort of adults with community-acquired pneumonia demonstrated that those with the A/A genotype were at greater risk for presenting with the clinical symptoms of sepsis [83]. Patients in postoperative and trauma intensive care units who developed sepsis and were homozygous for the LT-α+250 A allele have higher levels of TNF-α and a higher mortality rate [75,84,85]. Similarly, an association among the LT-α+250 A allele, higher serum levels of TNF-α, and higher mortality rate in bacteremic children has been observed [73]. Children who were heterozygous at this position (LT-α+250 GA) had an intermediate mortality rate. Thus, most evidence appears to support an association among certain genotypes in the regulatory region of the gene coding for TNF-α, levels of TNF-α production, and mortality rate of patients with sepsis.

Genetic polymorphisms in many other proinflammatory and antiinflammatory cytokines that influence the levels or function of the cytokines have also been examined to determine whether these genetic variations are associated with susceptibility to or outcome from sepsis (see Table 16.1). These include IL-1α, IL-1β, IL-1_{RA} [86–88], IL-6 [89–91], IL-8 [92–95], and IL-10 [96–99], and the list continues to grow rapidly as more polymorphisms in cytokine genes are discovered. Several of the above-mentioned cytokines contain polymorphisms that appear to be associated with sepsis. The reader is referred to a recent review for more detail [10].

Genetic polymorphisms within noncytokine genes have also been examined for the influence of genetic variations on critically ill patients. Heat shock proteins (HSPs) are a family of stress-inducible proteins expressed in response to heat as well as a number of other noxious stimuli, including endotoxin and other mediators of severe sepsis [100]. These proteins play an important role in cell survival during stress [101] and are involved in a number of important cellular functions, including the folding, assembly, and translocation of proteins across membranes [102,103]. Polymorphisms within the genes coding for HSPs that influence HSP production [104] have been shown to be associated with more organ system failure in trauma patients [105] and the development of septic shock but not mortality in adults with community-acquired pneumonia [106].

Angiotensin I–converting enzyme (ACE) is present in all tissues, particularly the pulmonary endothelium. Angiotensin I–converting enzyme is primarily responsible for converting angiotensin I to angiotensin II but is also involved in the metabolism of chemotactic peptides, suggesting that it may play a role in the inflammatory response. Individuals have been shown to have variable plasma and tissue levels of ACE, and evidence suggests that these variable levels are caused in part by genetic factors [107]. Specifically, an insertion (I)/deletion (D) of a 287 base repair repeat sequence in the noncoding intron 16 of the gene coding for ACE [108,109] is associated with variable plasma levels; individuals with the DD genotype have higher plasma and tissue levels of ACE than individuals who are heterozygous or are homozygous for the insertion sequence [110,111]. Association studies have suggested that the D/D polymorphism is associated with more severe meningococcal disease in children as measured by a higher predicted risk of mortality, greater prevalence of inotropic support and mechanical ventilation, and longer intensive care unit stay [112].

The pathogenesis of multiple organ system failure in sepsis is believed to involve in part endothelial dysfunction and intravascular fibrin deposition [113]. Diminished activity of anticoagulants, or elevated levels of inhibitors of fibrinolysis, can lead to fibrin deposition and may contribute to multiple organ system failure. Plasminogen activator inhibitor 1 (PAI-1) is an inhibitor of fibrinolysis because of its ability to inhibit the potent fibrinolytic, plasminogen activator. High plasma concentrations of PAI-1 have been observed in sepsis [114] and severe meningococcal disease [115], and high concentrations are correlated with worse outcome. A single nucleotide I/D polymorphism exists within the promoter region of the gene coding for PAI-1 that appears to influence the amount of PAI-1 production, with individuals homozygous for the 4G/4G genotype producing more PAI-1 than either individuals heterozygous (4G/5G) or homozygous for five guanines (5G/5G) [116]. Children with the 4G/4G genotype who had meningococcal disease had higher plasma levels of PAI-1 [117] and an increased risk of death from sepsis than did children with either the 4G/5G or the 5G/5G genotype [117–119]. This polymorphism is not only associated with outcome from meningococcal disease but appears to be a marker for poor outcome after severe trauma [120]. Thus, there appears to be a strong association between the 4G/4G genotype in the PAI-1 gene, high plasma concentrations of PAI-1, and worse outcome in critical illness.

In summary, there are a number of polymorphisms within genes involved in recognition of bacterial pathogens and response to bacterial pathogens that appear to be associated with the development of sepsis and its outcome (see Table 16.1). In a number of cases, changes in the level or the function of the gene's protein product has been documented.

Genetic Polymorphisms in Acute Respiratory Failure and Lung Injury

Respiratory failure is one of the major reasons for admission to both adult ICUs and PICUs. The causes of respiratory failure in children are too numerous to list here but include pulmonary causes such as ARDS, asthma, and bronchopulmonary dysplasia; infectious causes such as pneumonia and bronchiolitis; and neurologic causes such as central hypoventilation and ingestions. Thus, the list of potential genetic polymorphisms that may influence respiratory failure is likely to be very diverse. We discuss two examples of genes that play a role in respiratory physiology and inflammation for which data suggest that polymorphisms may influence the degree of respiratory failure and lung injury in critically ill patients.

Community-acquired pneumonia is a primary cause of respiratory failure in both children and adults. Although most individuals with community-acquired pneumonia have minimal lung injury, a small but significant number develop respiratory failure and severe lung injury. The most severe form of lung injury is ARDS and results in high morbidity and mortality in both children and adults. This variability in the degree of lung injury in patients with community-acquired pneumonia raises the possibility that genetic variation influences the susceptibility to and outcome from lung injury.

Genes coding for proteins involved in normal lung physiology, such as pulmonary surfactant, are ideal candidate genes in which genetic variation might influence the degree of lung injury and respiratory failure. Indeed, it has been suggested that alterations in surfactant may play important roles in these processes [121,122].

Surfactant contains four major proteins, A, B, C, and D, which exhibit a variety of functions, including a role in host defenses in the lung [123–128] and the reduction of surface tension at the air–liquid interface. The surfactant protein-A (SP-A) genes as well as SP-B, -C, and –D genes are polymorphic, and polymorphisms in these genes have been associated with lung disease [129–132]. The polymorphisms in the SP-A genes (A1 and A2) and SP-B gene are the best characterized, and many reports have shown association of these genes with lung disease [130,131,133–136]. We will discuss SP-B and how a common genetic polymorphism in the gene coding for SP-B may influence respiratory failure and lung injury in critically ill children.

Deficiency in, or impaired activity of, SP-B is implicated in a variety of interstitial pulmonary diseases, including acute respiratory failure and death in newborns and mice [137–139], increased sensitivity to hyperoxia [140], human congenital proteinosis [141,142], respiratory distress syndrome in premature infants [143,144], and ARDS [130,145,146]. Indeed, in patients with ARDS, both a lower level of surfactant proteins in bronchoalveolar lavage fluid [146,147] and a diminished ability of surfactant to lower surface tension have been found [148]. In addition, calfactant, a natural lung surfactant containing high levels of SP-B, improved oxygenation and decreased mortality in children with acute lung injury [149]. Genetic variations in the regulatory or functional regions of the gene encoding for SP-B may, therefore, influence susceptibility to, and outcome from, severe lung injury and respiratory failure.

The gene coding for SP-B is located on chromosome 2 and consists of 11 exons, including a 3′-untranslated sequence [150]. Surfactant protein-B is synthesized as a 381-amino acid precursor protein that is proteolytically cleaved to the active 79-amino acid form. Several SNPs within intron 2, exon 4, and the 5′ and 3′ flanking regions of the gene coding for SP-B have been identified. A C/T nucleotide variation at position 1580 in exon 4 changes amino acid 131 from threonine to isoleucine [141], altering a site for N-linked glycosylation [151,152]. Glycosylation of this site may impact the processing and/or function of SP-B [152,153] resulting in decreased functional SP-B. We have examined the genetic polymorphism in the SP-B+1580 site in adults with community-acquired pneumonia and demonstrated that a higher percentage of those individuals with the less common C/C genotype developed respiratory failure requiring mechanical ventilation and met the criteria for ARDS compared with those individuals with the T/C or T/T genotypes [154]. Whether or not this polymorphism is associated with more severe lung injury in children with community-acquired pneumonia is currently being investigated.

Another candidate gene in which polymorphisms might be associated with more severe lung injury is the gene coding for ACE. As mentioned earlier, ACE is present in the pulmonary endothelium and is responsible for converting angiotensin I to angiotensin II (ATII). In adults with ARDS, ACE concentrations in bronchoalveolar lavage fluid are elevated [155] as are the transpulmonary gradient and circulating concentrations of ATII [156]. An association between the D allele, which is associated with higher plasma tissue levels of ACE as described earlier, and ARDS has been observed in adults [157]. A higher percentage of adults with ARDS had the D/D genotype than did adults who were at risk for the development of ARDS, including those who underwent coronary artery bypass graft surgery or were in the ICU for other reasons. Thus, the genetic variation in the ACE gene may be associated with more severe lung injury.

Genetic Polymorphisms in Cardiovascular Surgery

Children who have undergone cardiovascular surgery represent a significant number of patients in pediatric and/or cardiovascular intensive care units. Whether genetic polymorphisms are associated with various complications in the post-operative period in this population is another area of intense research. One of the potential sources of many of the complications observed in the post-operative period may be the release of inflammatory mediators, including TNF-α, IL-6, IL-8, and ATII [158]. Studies of adults and children undergoing cardiovascular surgery involving the use of cardiopulmonary bypass have demonstrated a release of proinflammatory and antiinflammatory cytokines after surgery [159]. Various stimuli have been suggested to initiate cytokine release after cardiopulmonary bypass, including exposure of blood to the foreign surface of the bypass machine, complement activation [160,161], ischemia–reperfusion injury [162], and endotoxin released because of gastrointestinal tract hypoperfusion [163]. Whatever the inciting event, this inflammatory cascade may result in postoperative complications such as cardiovascular instability, systemic inflammatory response syndrome, and multiple organ dysfunction [164–166]. As discussed previously, genetic variation influences the levels of many of the proinflammatory and antiinflammatory cytokines. It is plausible, therefore, that the complications observed in patients after exposure to cardiopulmonary bypass may be, in part, influenced by genetic variation.

Few studies have examined the association between polymorphisms in genes involved in inflammation and complications in children who have undergone cardiac surgery, and, therefore, we will discuss studies examining these associations in adults who have undergone coronary artery bypass graft (CABG) surgery. Mechanical ventilation greater than 24 hours after CABG is considered prolonged and is a well-known complication of CABG observed in adults. Approximately 6% of patients undergoing their first CABG surgery and 11% of those undergoing repeated CABG surgery are unable to be tracheally extubated by 24 hours [167]. The etiologies for prolonged mechanical ventilation include both pulmonary-related (atelectasis, bronchospasm, congestive heart failure [CHF], ARDS, and acute lung injury) and nonpulmonary-related (cerebrovascular accident, cardiogenic shock, and excess postoperative bleeding) events. Understanding the underlying mechanisms resulting in prolonged mechanical ventilation might allow both identification of those patients at increased risk and development of therapies and strategies specific for such patients.

The associations between genetic polymorphisms located in genes coding for TNF-α, LT-α, IL-10, IL-6, and ATII and various complications after cardiopulmonary bypass have been studied [168–172]. We have examined the association between some of these polymorphisms and prolonged mechanical ventilation in adults who have undergone CABG surgery [173]. Adults with the A/A "TNF-α hypersecretor" genotype at either the TNF-α–308 or the LT-α+250 sites demonstrated overall shorter times to extubation and lower risk of prolonged mechanical ventilation. This appears to be contrary to the idea that hypersecretion of proinflammatory mediators may be detrimental to patients in the postoperative period [174,175]. Possible explanations for the apparent beneficial effect of TNF-α are that TNF-α appears to protect the myocardium from hypoxic insults [176,177] and that TNF-α stimulates protective HSPs [178–180]. Further studies are needed to better define the role of proinflammatory mediators in prolonged mechanical ventilation in this population.

Another potential mediator that could play a role in postoperative complications is ATII. Animal studies have demonstrated that ATII plays a role in myocardial ischemia–reperfusion injury [181] and contributes to depression of myocardial function [182]. Elevation of ATII may contribute to various causes of prolonged mechanical ventilation discussed earlier; for instance, its role in ischemia–reperfusion injury may contribute to postoperative ARDS/acute lung injury, whereas cardiac effects may increase postoperative CHF. As mentioned previously, ACE is present in the pulmonary endothelium and converts ATI to ATII. Concentrations of ACE are elevated after CABG surgery [183], and these elevated concentrations appear to be influenced in part by genetic polymorphisms. Other studies have demonstrated that adults with the D allele had higher mortality and restenosis rates after CABG surgery compared with patients with the I allele [184]. Also, as mentioned previously, the D allele appears to be associated with susceptibility to and prognosis of ARDS [157], an important cause of prolonged mechanical ventilation in the postoperative period for adults who have undergone CABG surgery. The ACE D allele is also associated with prolonged mechanical ventilation in this population except for patients who had their CABG procedure off-pump [185]. This observation suggests that the off-pump approach for those patients with the D/D genotype who are at higher risk for prolonged mechanical ventilation may decrease the incidence of this complication.

The risk for prolonged mechanical ventilation in the CABG population (like many of the conditions treated in the ICU) may be influenced by multiple proteins and their genetic polymorphisms. Currently there are an increasing number of studies examining the association of genetic polymorphisms in multiple genes with certain clinical conditions. The associations of the I/D polymorphism in the ACE gene and the TNF-α–308 and LT-α+250 polymorphisms with the risk of prolonged mechanical ventilation in the CABG population have been analyzed. Individuals with the G/G haplotype at TNF-α–308 and LT-α+250 loci and the D/D polymorphism of the ACE gene had a significantly higher adjusted hazards ratio than did individuals who did not have the G/G haplotype at TNF-α–308 and LT-α+250 loci and had the I/I polymorphism of the ACE gene [185]. Thus, data are beginning to suggest that some of the postoperative complications observed after cardiopulmonary bypass may be influenced by genetic variation in the host. In addition, the possible influences of genetic variation in receptors and/or components of the signal transduction pathways of the various inotropic agents, vasoconstrictors, and vasodilators used in the care of children who have undergone cardiovascular surgery are also beginning to be analyzed.

Genetic Polymorphisms in Thrombosis

Thromboses in both arteries and veins are significant problems in children in PICUs [186–192]. These children are exposed to multiple risk factors for thrombosis, including sepsis and central venous catheters, with reports of deep venous thrombosis in 7.5% to 50% of children with central venous catheters [193–196]. A number of inherited defects in the coagulation and thrombolytic systems also predispose children to thrombosis [197–199]. These defects can result in hyperactive coagulation, hypoactive anticoagulation, or hypoactive fibrinolysis. Several genetic variations (Table 16.2) have been identified in genes coding for components of the coagulation system that influence the quantity or function of these proteins and have been shown to be significant risk factors for thrombosis [198,200]. These include variations in genes coding for factor V [201], prothrombin [202–205], antithrombin [206–210], protein C [211–215], protein S [216–221], methylentetrahydrofolate reductase [222], endothelial nitric oxide synthase [223–225], α-fibrinogen [226–229], and factor XIII [228,230–235]. However, no researchers have reported the relative risks of these various genetic polymorphisms in the development of thrombosis in children in PICUs. The Arg506Gln polymorphism in the factor V gene (factor V Leiden) has been reported in 13%–45% of pediatric patients with thromboembolism [236–239], but this population may not necessarily reflect the population of PICU patients who also have the other nonhereditary risk factors mentioned earlier. Because the development of thrombosis can be deterred with anticoagulants, knowledge of a child's genetic polymorphisms in the genes coding for components of the coagulation system might identify children who could benefit most by anticoagulant therapies.

Table 16.2. Genetic polymorphisms examined for associations with risk of thrombosis.

Gene	Polymorphism*	Consequence of polymorphism
Factor V	G1691A; Arg506Gln; (factor V Leiden)	Resists activated protein C
Prothrombin	G20210A (in 3′-UTR)	Increased levels; associated with risk of deep venous thrombosis
Antithrombin	Multiple sites	Decreased levels and activity
Protein C	Multiple sites in promoter	Decreased levels; associated with risk of venous thrombosis
Protein S	Multiple sites	Decreased levels; increased thrombosis
Fibrinogen	Thr312Ala	Affects structure/function and FXIII cross-linking; associated with pulmonary embolism
Methylentetrahydrofolate reductase	C677T; Val/Ala	Decreased enzymatic activity; increased levels of homocysteine; associated with arteriovenous fistula
Endothelial nitric oxide synthase	G894T; Glu298Asp	Less stable enzyme; associated with restenosis of stents; associated with myocardial infarcts
Factor XIII A	Val34Leu	Increased cleavage and activation; associated with deep venous thrombosis

*The terms used for the various polymorphisms are the ones most commonly used in the literature and may refer to the nucleotide position, amino acid position, or name of the allele. This table is representative of polymorphisms examined in thrombosis but does not include all such polymorphisms.

Pharmacogenomics

Another area of pediatric critical care in which genetic polymorphisms influence critical illness is pharmacology. Pharmacogenomics attempts to determine the genetic factors that affect the various aspects of drug action, including drug transport, binding to receptors and signal transduction, and metabolism. That genetics can influence some drug responses was first suggested by associations between inheritance or ethnicity and abnormal drug responses and further defined through biochemistry and molecular genetics [for review, see refs. 240–243]. While the list of genetic polymorphisms in genes coding for drug transporters, receptors,

and enzymes involved in drug metabolism is growing rapidly [244], there are very few examples of genetic polymorphisms that influence the action of drugs commonly used in PICUs.

The best-described examples of genetic polymorphisms that influence drug response are those that are found in genes coding for enzymes involved in drug metabolism (Table 16.3). One example is briefly described here in order to demonstrate the clinical relevance of such genetic variations. Thiopurine S-methyltransferase (TPMT) is an enzyme primarily responsible for inactivation of the thiopurines mercaptopurine and azathiopurine used as immunosuppressants and chemotherapeutic drugs. Genetic polymorphisms in the gene coding for TPMT result in a nonfunctioning enzyme; thus, patients receiving mercaptopurine or azathiopurine who inherit the nonfunctional allele accumulate high concentrations of the active metabolites and are at risk for developing life-threatening hematopoietic toxicities [245–247]. Clinical diagnostic tests are available for detecting the SNPs in the TPMT gene that result in TPMT deficiency, thereby allowing for the identification of patients at high risk for thiopurine toxicities. Patients receiving mercaptopurine or azathiopurine who are genetically predisposed to be TPMT deficient have been treated successfully for their oncologic diseases using approximately 5%–10% of the conventional dose of the thiopurines [245,246] without the toxicities. This represents a good example of modifying drug therapies based on an individual's genetic makeup.

A second example of a genetic variability that may influence drug action involves the β_2-adrenergic receptor (β_2-AR). β_2-Adrenergic receptor agonists are the most potent bronchodilators and continue to be the mainstay treatment for exacerbations of asthma [248]. β_2-Agonists activate the β_2-AR, resulting in coupling of the receptor–agonist complex to G_s, which in turn activates adenylate cyclase and increases the intracellular production of cyclic AMP (cAMP), resulting in the dilation of the smooth muscle lining the small bronchiolar airways [249]. Substantial variation in β_2-AR response between individuals has been observed [250]. Over the past several years many studies have examined the possibility that alterations in β_2-AR function might be associated with asthma, severity of asthma, or asthma phenotypes.

A number of SNPs within the gene coding for the β_2-AR have been identified [251]. An SNP upstream of the coding region (−47 C/T) appears to be associated with the regulation of β_2-AR expression in the cell [252,253]. In addition, the two most common SNPs, glycine or arginine at position 16 (Gly16 or Arg16) and glutamic acid or glutamine at position 27 (Glu27 or Gln27), alter the amino acid sequence of the β_2-AR, which in turn alters properties of the receptor [251]. More recently, Drysdale et al. [254] genotyped 13 SNPs in ~80 individuals and identified 12 different combinations of these individual SNPS in the β_2-AR gene, meaning that there are 12 β_2-AR haplotypes (and hence alleles). Only one haplotype has Glu at amino acid 27; this is also the only haplotype with C at the −47 polymorphic site [254]. The β_2-AR variant with C at −47 has been shown to express lower levels of the receptor than variants with T at that site [252,253].

Studies have investigated the association of β_2-AR SNPs with asthma, asthma phenotype, or treatment modalities. Such studies have been performed primarily with single SNPs in the adult Caucasian population. Although there is no strong evidence linking a specific β_2-AR genotype to asthma, there are a number of studies linking specific genotypes to asthma phenotypes [255–260]. In children there is an association between the homozygous Gly16 genotype and bronchodilator desensitization [261], and we have recently reported an association of the Gln27Glu genotype with the need for aminophylline treatment in African-American children with status asthmaticus [262]. Aminophylline inhibits phosphodiesterase, the enzyme responsible for degradation of cAMP, and consequently the level of β_2-AR–stimulated cAMP is greater and its degradation is delayed, prolonging the elevated cAMP levels in the cell. These results suggest that African-American children with this genotype may have diminished response to β_2-agonist therapy and may respond more effectively to treatment with a phosphodiesterase inhibitor in addition to the β_2-agonist therapy. One possible explanation for the association of the Gln27Glu genotype with aminophylline treatment is that these patients may have a lower β_2-agonist–stimulated cAMP response than patients with the Gln27Gln genotype, and aminophylline addition may be required to increase cAMP to levels that are clinically efficacious. Presumably the Glu27 variant is responsible, as the need for aminophylline treatment is seen only in patients who have this variant of the receptor. A lower cAMP response could be caused by lower expression of the Glu27 variant, which has been reported in in vitro studies [252,253], or by increased desensitization of the β_2-AR Glu27 variant, which is still controversial as different studies have concluded that the Glu27 variant undergoes greater [263] or lesser [264] desensitization than the Gln27 variant.

Table 16.3. Genes in which polymorphisms alter drug effects.

Gene*	Specific drug or drug class	Consequence of polymorphism
β_2-Adrenergic receptor	Albuterol, terbutaline	Decreased bronchodilation
α_1-Adrenergic receptor	α_1-Agonists	Decreased cardiovascular response to α_1-agonsts
G_s protein β	β-Blockers	Decreased antihypertensive effect
ALOX5	Leukotriene receptor antagonists	Decreased effect on FEV_1
Serotonin transporter	Antidepressants	Decreased clozapine effects, decreased antidepressant response
CYP2C9	Warfarin, phenytoin, nonsteroidal antiinflammatories	Increased anticoagulant effects of warfarin
CYP2D6	Antidepressants, codeine, β-blockers	Decreased codeine analgesia, increased antidepressant toxicity
CYP3A4/3A5/3A7	Midazolam, steroids, calcium channel blockers	Altered clearance of midazolam and steroids
CYP2C19	Omeprazole	Altered peptic ulcer response to omeprazole

*This table is representative of genes in which genetic polymorphisms have been shown to alter drug effects but does not include all such genes and their polymorphisms.

Limitations

Association studies attempting to examine the influence of genetic polymorphisms in specific diseases have several limitations that are important to keep in mind when reading the literature. A few of these limitations are briefly discussed here, and the reader is referred to a more comprehensive review of these limitations [265]. First, it is important that the correct control population is used in

the study. For example, in some sepsis studies the frequency of a polymorphism in the group of patients with sepsis is compared with the frequency of the polymorphism in a healthy control population. However, healthy individuals are not the appropriate control population, as they may not have been exposed to the same pathogens to which the patients with sepsis were exposed. A more appropriate control group for comparison would be a group of patients with a similar infection who did not develop sepsis.

A second limitation is that in many studies subjects within the study and control groups are from various ethnic groups. It is now well known that the frequency of many of these polymorphisms varies between ethnic groups and so comparisons should only be made within ethnic groups. Finally, the specific nucleotide variation being investigated may in fact not be directly involved but rather closely linked to the actual gene responsible for the effect.

Conclusion

In summary, there is little doubt that host genetic variation is responsible for some of the variable disease presentation, response to therapy, and final outcome observed in critically ill children. Identification of genetic polymorphisms that will ultimately be useful in identifying critically ill children at increased risk will allow for a more individualized approach to therapy. Carefully controlled studies examining candidate genes alone and in combination with other genes will be required to determine whether patient treatment can be tailored more specifically to an individual patient's genetic makeup.

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