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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...
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.

**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