Molecular genetics is the study, at the molecular level, of how genetic information is stored, inherited, and expressed and of how it influences the structure and function of cells in health and in disease. Although molecular approaches have been used for decades in the laboratory and are at the core of modern medical education, they are only now beginning to influence clinical practice. A variety of sophisticated techniques permit rapid and affordable DNA sequencing, gene expression profiling, gene cloning, gene manipulation, gene transfer, recombinant protein production, and other technologies of enormous biomedical importance. Success in genomics has spawned additional ambitious endeavors, including proteomics, pharmacogenomics, and bioinformatics. These techniques are providing new diagnostic, prognostic, and therapeutic opportunities in all areas of medicine, including anesthesiology. With the use of molecular criteria and the diminishing cost of analytic technologies, anesthetic practice will become more individualized, and greater emphasis will be placed on the patient’s genetic makeup. Both surgical and nonsurgical decisions will increasingly accommodate molecular data crucial to perioperative anesthetic management. In this article we have summarized three lectures on congenital malformations, pharmacogenetics, and proteomics presented at the 22nd Annual Meeting of the Society for Pediatric Anesthesia. (Anesth Analg 2010;111:1264–74)

PHARMACOGENETICS

*Primum non nocere*—“First, do no harm”—remains a central tenet in the practice of medicine. As anesthesiologists, we administer extremely potent and potentially lethal drugs with narrow therapeutic margins to patients who span the spectrum of age, disease, and health. Indeed, the margin of safety of most of the drugs used in daily anesthetic practice is so narrow that for generations, anesthesiology was among the most dangerous and highest-risk professions in medicine. Few other specialties use drugs, such as neuromuscular blocking drugs, that when given in appropriate therapeutic doses are lethal unless the patient is rescued by timely airway and ventilatory management. Incredibly, despite these inherent dangers, we have over a relatively short period of time improved the safety and quality of our craft to the point that it is now among the safest in all of medicine. Indeed, through better training, vigilance, and the use of new and improved drugs and monitoring devices, anesthesia is the only field of medicine approaching six-sigma quality.

The daily use of anesthetics in millions of patients has provided practitioners with a keen sense of how anesthetic drugs behave in the various populations we serve. However, how anesthetic drugs work is based on population studies; how individuals respond is not as clear. Individual response is variable and remains a primary cause of adverse drug events across the entire drug spectrum.
Understanding the science behind drug and individual patient variability can help us develop therapies and guidelines to avoid these adverse drug reactions and the harm associated with them.

In genetic pediatric diseases, genetic variability is frequently evident as an outward physical manifestation. In pharmacogenetics, genetic variability is not something that is visible or palpable. Each individual has a unique genetic code. How an individual’s genetic code affects drug action or a clinical response is the basis of the science of pharmacogenetics. The aim of the science of pharmacogenetics is an individualization of drug therapy to increase drug efficacy and decrease drug toxicity.

Pharmacogenetics seeks to link differences in gene structure or genotype (polymorphisms) with pharmacologic differences in drug action (phenotype). A polymorphism can exert an effect on a drug or drug action anywhere in the body where a drug goes or acts (Fig. 1). Polymorphisms can be as small as a substitution of a single nucleotide base pair on an entire chromosome to multiple inherited linked base pair changes (a haplotype). Individually, a single polymorphism that alters absorption, metabolism, drug transport, or receptor site can have a profound effect on a single drug’s action.

For a classic illustration of a single nucleotide polymorphism (SNP) causing a profound effect, we can look to one of the earliest described pharmacogenetic discoveries. In the 1950s Kalow and Gunn described prolonged apnea and muscle relaxation after the administration of succinylcholine. They ascribed their finding to a defect in the metabolizing enzyme butyrylcholinesterase. We now know this as pseudocholinesterase deficiency, and we know it is caused when a patient has 2 identical uncommon copies (1 from each parent, creating a homozygous recessive trait) of a single variant nucleotide substitution on each matching DNA strand.

Unfortunately, having a single polymorphism explain large individual differences in drug action is more the exception than the norm. Multiple polymorphisms in absorption, metabolism, drug transport, or a receptor site often combine, making it difficult to determine why an individual has an altered drug response. Take the example of morphine. An individual can possess a polymorphism that decreases the rate of metabolism of morphine (creating more free drug), have a polymorphism that decreases the rate of transport into the brain (decreasing drug availability to the receptor), and finally have a polymorphism that makes the receptor less sensitive to morphine (decreasing sensitivity). The end result is that the patient has an effect within normal limits, but he or she still possesses a profoundly nonstandard genotype for each major factor in drug effect.

What can make analysis of drug action even more complex are epigenetic factors. This is beyond the scope of this review but is worth mentioning because epigenetic factors can also cause profound alterations of drug action. Epigenetics is the study of changes in gene function that occur without a change in an individual’s DNA sequence. An example of epigenetic variability can be seen in sickle cell disease and how opioids work. All patients with sickle cell disease share the same genetic defect, yet some are rarely ill and others present with revolving-door episodes of vasoocclusive crisis. Why? Epigenetic studies are being used to answer this fundamental question.

As technology has grown, so has our understanding of the interaction between individual genetic differences and drug action. This has increased our awareness that previously believed “idosyncratic reactions” are actually related to individual differences in genotype. In this section we will examine the common genetic polymorphisms that affect the day-to-day practice of the anesthesiologist from preoperative to postoperative periods.

Nomenclature
To discuss pharmacogenetics it is important to understand the language used to describe genetic polymorphisms. SNP is a term used to describe a single base pair substitution on a chromosome. There are 2 purine bases, adenine (A) and guanine (G), and 2 pyrimidine bases, cytosine (C) and thymine (T), in DNA. The purines and pyrimidines are paired together, A with T and G with C. When we discuss a SNP we are saying that one base pair has been substituted for another. For example, an A residue can be replaced by a G, C, or T, and the corresponding T will also be replaced by the matching base, as was noted above. When a SNP is described, generally the number in the nomenclature refers to the base pair location on a specific part of a chromosome. The first letter is the wild type or “normal” base pair at that location; the second letter is the variant or “abnormal” base pair. Thus, A118G, 118A/G, and 118A>G all refer to a substitution of a guanine base for an adenine base at the 118th base pair on a specific locus of a chromosome or gene.

Another way that polymorphisms are coded is by amino acids. Each 3 base pairs uniquely codes for a different amino acid; for example, GAT codes for aspartic acid (Asp). If you change one base pair and it becomes GAA, this will now code for glutamine (Glu). The nomenclature when referring to amino acids for this change would be Asp40Glu or a Glu substituting at the 40th amino acid to a locus for an Asp.

Liver enzymes are also referred to by a specific nomenclature. The cytochrome P450 system is generally referred to as CYP450 or just CYP. This is followed by the enzyme designation, such as 3A4 or 2D6. Polymorphisms of these cytochromes are designated by the order in which they were discovered. For example CYP3A4*5 is the fifth variant of the CYP3A4 system that is described. In this system the *1 allele is the wild type (normal) gene. This provides less
specific detail than does the nomenclature, in which specific substitutions and locations are noted but is nonetheless standard for the CYP polymorphisms.

**Drugs Commonly Used in Anesthetic Practice**
The great majority of drugs that are given in anesthesia practice have different effects in different people. To best summarize this, Table 1 describes many of the known relevant polymorphisms that affect opioid drug action. Table 2 provides detail on other examples of clinically relevant drugs to daily practice and commonly seen pharmacogenetic implications. From these tables it is clear that often it can be difficult to characterize variant responses of drugs by single polymorphisms.

**Codeine**
Problems related to opioid use and pharmacogenetics having to do with the parent drug codeine have been well characterized. Codeine is a parent or “prodrug” drug because it has minimal clinical effect until it is metabolized in the liver (about 10% of drug) by CYP2D6 to morphine. Up to 10% of the population in this country are poor metabolizers of codeine and get mimimal analgesic effect from this drug. In children, because of both this polymorphism and other maturation factors, the number of patients who get no analgesic benefit from morphine may be as high as 36%. This may be caused by the low amount of drug absorption or additional polymorphisms reducing overall effectiveness.

A small population of individuals, most commonly seen in persons of East African descent, are ultrarapid metabolizers. These individuals convert the majority of codeine into morphine. For these patients, respiratory depression and apnea are very real possibilities after even a single dose of drug. This is of particular concern in nursing mothers. In a recent case report, an infant died secondary to the use of codeine in a mother who was an ultrarapid metabolizer for CYP2D6, and with a variant gene that decreased her

---

### Table 1. Polymorphisms That Affect Opioid Drug Action

<table>
<thead>
<tr>
<th>Drug target affected</th>
<th>Gene</th>
<th>SNP</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opioids* (i.e., morphine)</td>
<td>Mu-1 receptor</td>
<td>OPRM1</td>
<td>A118G</td>
</tr>
<tr>
<td>Opioids* (i.e., morphine)</td>
<td>Intracellular signaling</td>
<td>Beta-arrestin 2</td>
<td>A304G/C8622T</td>
</tr>
<tr>
<td>Codeine</td>
<td>Liver metabolism: N-demethylation</td>
<td>CYP2D6</td>
<td>G1846A</td>
</tr>
<tr>
<td>Codeine</td>
<td>Liver metabolism: N-demethylation</td>
<td>CYP2D6</td>
<td>3 copies of CYP2D6 gene</td>
</tr>
<tr>
<td>Methadone</td>
<td>Liver metabolism: N-demethylation</td>
<td>CYP2D6</td>
<td>G1846A</td>
</tr>
<tr>
<td>Tramadol</td>
<td>Liver metabolism: N-demethylation</td>
<td>CYP2D6</td>
<td>G1846A</td>
</tr>
<tr>
<td>Morphine</td>
<td>Liver metabolism: glucuronidation</td>
<td>Uridine diphosphate-glucosyl transferase</td>
<td>C-161T and C802T</td>
</tr>
<tr>
<td>Morphine</td>
<td>Drug transport</td>
<td>MDR1/ABCB1 and ABCTT</td>
<td>C3435T and G2677T/A</td>
</tr>
</tbody>
</table>

**Table 2. Polymorphisms That Affect Perianesthetic Drugs**

<table>
<thead>
<tr>
<th>Drug target affected</th>
<th>Gene</th>
<th>SNP</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedative hypnotics</td>
<td>Drug metabolism</td>
<td>G681A</td>
<td>CYP2C19</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Immune response</td>
<td>Human leukocyte antigen</td>
<td>HLA-DRB1*11</td>
</tr>
<tr>
<td>Nonsteroidal analgesics</td>
<td>Drug metabolism</td>
<td>CYP2C9</td>
<td>A1075C</td>
</tr>
<tr>
<td>Ibuprofen, Naproxen, Celecoxib</td>
<td>Immune response</td>
<td>Human leukocyte antigen</td>
<td>HLA-DRB1*11</td>
</tr>
<tr>
<td>Antiemetics</td>
<td>Drug metabolism</td>
<td>CYP2D6</td>
<td>3 copies of CYP2D6 gene</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>Drug metabolism</td>
<td>CYP2D6</td>
<td>G1846A</td>
</tr>
<tr>
<td>Cardiovascular drugs</td>
<td>Drug receptor</td>
<td>B-1 receptor</td>
<td>49Ser389Gly/49Ser389Gly</td>
</tr>
</tbody>
</table>

SNP = single nucleotide polymorphism.

---

*This assumption is based on studies regarding the mu-1 receptor and beta-arrestin using morphine and fentanyl.
metabolism of morphine.\textsuperscript{5} In this age of multiple oral opioid choices, it makes little sense to use a drug as variably ineffective and potentially dangerous as codeine.

\textbf{Morphine}

The drug action of morphine, a metabolite of codeine as is described above, is very difficult to describe in relation to a single-gene hypothesis. To understand the action of morphine in relation to individual genetics, it is important to follow morphine as it enters the body and gets absorbed and moves to its site of action.

After an IV injection, morphine is metabolized in the liver. Multiple liver enzymes influence the metabolism of morphine. Morphine glucuronidation is the major path of first-pass metabolism of morphine. An alteration in the genotype of uridine diphosphate glycosyl transferase (UDGT) causes increased glucuronidation of morphine. Theoretically, this should cause a decrease in morphine’s effectiveness.

After this first-pass metabolism, morphine is transported across cell membranes to the brain by the ATP-binding cassette (ABC) transporters, specifically the multidrug resistant (MDR1)/ABCB1 efflux transporter (methadone, fentanyl, and meperidine are also transported by the same mechanism).\textsuperscript{6} Research into transporter polymorphisms is just beginning. However, early results show that polymorphisms of this transporter can result in as much as a 2-fold increase in opioid requirements for patients with 2 copies of relevant polymorphisms.

After transport, morphine has now reached its site of action, the \( \mu \)-opioid receptor (OPRM). The OPRM-1 gene codes for this receptor, and 1 polymorphism of this gene (A118G) results in a decreased sensitivity to opioids.\textsuperscript{5} To have effect, the \( \mu \)-1 opioid receptor at this point needs to send a signal to the brain to activate analgesic effect. Beta arrestin is this internal signal. The common variant polymorphism coding for \( \beta \) arrestin, C862T, increases morphine effectiveness.\textsuperscript{6} This work is very preliminary, and more research is being currently generated to better elucidate both the receptor and drug signal relationships. The complexity of the relationship of these 4 target sites and their interaction together represents the future of opioid pharmacogenetic research.

\textbf{Volatile Anesthetics}

Individual sensitivity to volatile anesthetics on the basis of genetics has not been as well studied or defined as with the opioids. As was seen in the research by Liem et al.,\textsuperscript{7} many redheads have distinct polymorphisms in the melanocortin-1 receptor, which makes them less sensitive to desflurane (require more drug to achieve minimum alveolar concentration [MAC]). It has also been shown that ethnicity may play a role in MAC with Caucasian Jews having a MAC for sevoflurane of 2.4, Oriental Jews a MAC of 2.1, and European Jews a MAC of 1.9.\textsuperscript{8} Other than these studies and work on malignant hyperthermia (MH), there is little to show that any genetic variability will cause a change in sensitivity to volatile anesthetics. This is true even for halothane hepatitis despite halothane metabolism being primarily mediated by a single liver enzyme, CYP2E1.

MH is an autosomal dominant inherited myopathy associated with abnormal intracellular calcium release in skeletal muscle upon exposure to triggering substances such as volatile anesthetics and succinylcholine. There is no single polymorphism in the ryanodine 1 receptor that is singularly “the link” to this disease. There are 170 variations in this receptor that are linked to MH susceptibility.\textsuperscript{9}

\textbf{Muscle Relaxant}

The atypical variant polymorphism of succinylcholine metabolism is one of the first described and most well known pharmacogenetic anomalies. Plasma pseudocholinesterase (butyrylcholinesterase) deficiency decreases succinylcholine inactivation in 1 in 2500 individuals. People who are homozygous for this polymorphism (2 copies of the gene polymorphism of the allele butyrylcholinesterase Asp70Gly) have prolongation of drug action up to 60 times normal. Heterozygotes (those with 1 copy of the polymorphism) occur with a frequency of 1 in 25 in Caucasians but are rare in both Asians and African Americans. Heterozygotes have an increase in drug duration 3 to 8 times.\textsuperscript{10,11} This same deficiency affects the metabolism of mivacurium. Patients with fully functional plasma pseudocholinesterase take, on average, 30 minutes to recover from mivacurium. This recovery time is increased by 15 to 30 minutes in heterozygotes and up to 6 to 8 hours in homozygotes. There are other less well known allelic variants of plasma pseudocholinesterase.\textsuperscript{11} The majority of these are rare and lack standard tests to detect, but should be considered with altered response to succinylcholine.

\textbf{Clinical Pharmacogenetics}

Recent research with the anticoagulant drug warfarin serves as a good example of how to apply pharmacogenetics in a clinical setting. Warfarin is primarily metabolized in the liver by CYP2C9. Polymorphisms in the CYP2C9 gene result in very low dose requirements for warfarin. Genetic variation in the vitamin K system (VKORC1) also alters warfarin response. These 2 polymorphisms explain much of the variability of warfarin drug dosing in certain populations. In a recent study, polymorphisms in both of these genes were tested (a lab process that took about 2 hours) before initiation of drug therapy. Using information from these tests to establish an initial dose regimen resulted in 83% of patients reaching therapeutic, stable international normalized ratios within 2 weeks.

Applied pharmacogenetics is still in its infancy. Technology has not quite made it to the point at which all of the above genotypes can be analyzed before surgery and thus anesthetic management can be planned accordingly.

\textbf{PROTEOMICS}

The decoding of the human genome has fundamentally changed and retooled how anesthesiologists think and how new therapies will be brought into practice. Increasingly, the discovery, design, and evaluation of the drugs that are or will be used in the perioperative environment are based on genomics and individual genomic variation.\textsuperscript{12,13} Furthermore, much of these discoveries are dependent on the elucidation of protein mechanisms involved in both health and disease because proteins are at the heart of human physiology and pathophysiology. Proteins are the essential building blocks of cellular infrastructure, membrane receptors, ion channels, chemical messengers, and enzymes.
The Proteome and the Genome

Each of our cells contains all the genetic (DNA, RNA) information necessary to make a complete human being (“genome”). However, not all genes are expressed in all cells. Genes that code for enzymes essential to basic cellular function (e.g., glucose metabolism) are expressed in virtually all cells, whereas those with highly specialized function are expressed in only specific cell types (e.g., rhodopsin in retinal pigment epithelium). Expression of genes also changes in health and in disease as well as throughout development.\textsuperscript{12–15} The central dogma of modern biology—namely, that genes (DNA) are transcribed into messenger RNA (mRNA), which is translated into polypeptides on ribosomes—appears straightforward and simple. Unfortunately, it is not. Although there is only one relatively constant genome consisting of approximately 30,000 human genes, there are several million proteins. Why?

Any protein, though a product of a single gene (DNA transcribed to RNA and translated into proteins), may exist in multiple forms that vary within a particular cell or between different cells. In most cases, the initial polypeptide translation product undergoes some type of modification before it assumes its functional role. These posttranslational modifications encompass a wide variety of reversible and nonreversible chemical reactions. At their cellular destinations, proteins perform many functions that are often controlled by further posttranslational modification (e.g., phosphorylation of serine, threonine, or tyrosine), which may activate or inactivate enzymes, alter protein–protein interactions, change protein structure, or target proteins for degradation. Indeed, most proteins exist in several modified forms. These posttranslational modifications encompass a wide variety of reversible and nonreversible chemical reactions that affect protein structure, localization, function, and turnover. Finally, protein expression is variable in different parts of the body and may be affected by age, environmental conditions, and disease.\textsuperscript{12–15}

Proteomics is the science and methodology of the investigation of the proteome and is complimentary to genomic approaches that investigate DNA and RNA.\textsuperscript{12–16} In essence, proteomics is a massive scale-up of previous efforts and abilities at identifying individual proteins. Unlike protein chemistry in which individual proteins are identified, sequenced, and modeled to explore their structure and function, proteomics is the study of multiprotein systems, in which the focus is on the interplay of multiple, distinct proteins in their roles as part of a larger system or network. Analysis is directed at complex mixtures. Identification is not by complete sequence analysis but instead by partial sequence analysis with the aid of database matching tools. The context is systems biology, rather than structural biology.\textsuperscript{12,16} In other words, the point is to characterize the behavior of the system rather than a single component. Understanding the principles of protein–protein interactions, receptor signal transduction pathways, and enzyme catalysis is the central theme of nearly all biomedical research and is indispensable to modern anesthetic practice.

Tools of Proteomic Research

Analytical protein identification is built around a basic essential fact: most peptide sequences of approximately 6 or more amino acids are largely unique and can be used to identify the gene product, that is, the protein it came from. This identification is made by finding the peptide sequence match in a database of protein sequences. Furthermore, if several peptide sequences map to the same protein, the validity of the match is intensified.

Most analytical proteomic studies begin with a protein mixture, containing proteins of varying molecular weights and solubilities. The source can be a piece of tissue, cultured cells, a flask of bacteria, etc., and in proteomic research, the goal is to recover as much of the protein as possible. After separation and elimination of contaminants with detergents (SDS), reductants (DTT), denaturing agents (urea), and enzymes (DNase, RNase), proteins are separated most commonly in 2-dimensional (2-D) polyacrylamide gel electrophoresis (gels). Two-D gels separate proteins in 2 axes on the basis of charge (isoelectric focusing) and size.\textsuperscript{17} Individual spots on a gel represent a single protein or a small number of proteins.\textsuperscript{16} In contrast, in a 1-dimensional gel electrophoresis (Western blot), each band contains hundreds or thousands of different proteins.

Once separated, proteins are cleaved into peptides and analyzed by mass spectrometry (MS).\textsuperscript{18,19} Unlike DNA, proteins are inherently more complex and are not amenable to efficient replication. In proteomic research, the amounts of protein are limited. Thus, the MS instruments that measure proteins are sensitive enough to identify femtomole (10 to 15 mole) quantities of peptides or less. Furthermore, they must have the resolution to distinguish ions of similar mass/charge (m/z) ratios. Indeed, most instruments can distinguish differing m/z values of at least 1 Dalton, that is the mass of a single hydrogen atom. Finally, the measured values for the peptide ions or fragments identified by MS must be as close as possible to the values used to identify peptides in the databases because once the peptide mass is measured by the MS (usually to 4 decimal places), this mass number works as does a fingerprint to allow its identification in a peptide database found on the World-Wide Web. Hundreds of protein identifications can be performed in 1 day.

The most common methods of MS in proteomic research are matrix-assisted laser desorption time of flight (MALDI-ToF), electrospray ionization, surface-enhanced laser desorption time of flight (SELDI-ToF), and tandem MS/MS.\textsuperscript{18,19} Regardless of the MS device used, all of these instruments have 3 essential parts, namely a source that produces ions from the sample, a mass analyzer that resolves ions on the basis of m/z ratio, and a detector that detects the ions resolved by the mass analyzer.

Future Clinical Applications

The use of proteomics as a method of discovery in acute and chronic pain management and in drug-response phenotype is in its infancy but nevertheless exciting.
Neuropathic Pain

Neuropathic pain is a common syndrome that results from disease or dysfunction in the nervous system, such as from peripheral nerve or spinal cord injury. It is characterized by spontaneous ongoing or intermittent burning pain, an exaggerated response to painful stimuli, and pain in response to normally innocuous stimuli. Despite considerable research into the neurobiological mechanisms of neuropathic pain, our understanding of this disorder is still incomplete, and its treatment with current drugs—such as antidepressants, anticonvulsants, opioids, and nonsteroidal antiinflammatory drugs—is often inadequate.

Peripheral nerve injury is a common research model that produces neuroadaptive changes of cellular signals in the dorsal horn; such changes are thought to contribute to the central mechanisms that underlie neuropathic pain. After peripheral nerve injury, global changes of gene expression have been demonstrated by cDNA (complimentary DNA) microarray in the dorsal horn of the spinal cord. Some translational protein products have been further confirmed by Western blot and immunohistochemistry. However, these studies confirmed only the expression changes of some selected genes of interest at the protein level. It is possible that peripheral nerve injury could alter the expression of some genes without affecting their protein expression. In addition, peripheral nerve injury might modulate posttranscriptional regulation of some proteins without affecting gene expression. The global neuroadaptive changes of cellular signaling proteins in the dorsal horn under neuropathic pain conditions are still unclear.

Proteomic analysis can provide expression profiles of proteins and their posttranslational modifications in cells, tissues, and organs. Using proteomic approaches, Lee et al. first reported on 5 proteins that displayed differential expression in the spinal cord after spinal nerve injury. Kunz et al. also found 5 regulated proteins in the spinal cord after chronic constriction injury. However, many important classes of proteins that undergo significant expression changes in the dorsal horn under neuropathic pain conditions were not identified in those proteomic studies. Furthermore, other proteins may not be identified secondary to too-low levels or difficulty in analysis of hydrophobic proteins. Therefore, more in-depth proteomic analysis is required to study nerve injury–induced global changes in protein expression in the spinal dorsal horn.

We recently demonstrated that peripheral nerve injury alters the expression and subcellular distribution of some specific dorsal horn proteins that are involved in transmission and modulation of noxious information, cellular metabolism, plasma membrane receptor trafficking, oxidative stress, apoptosis, and degeneration under neuropathic pain conditions. Now that these proteins have been identified via proteomic analysis, hypothesis-driven studies can be developed to understand the central mechanism that underlies the maintenance of neuropathic pain.

Opioid Tolerance

Opioid tolerance is defined by a reduced responsiveness to μ-agonist opioids such as morphine or fentanyl and is usually manifested by the need to use increasing doses to achieve the desired effect. Tolerance limits the analgesic efficacy of opioids and also contributes to the social problems surrounding abuse. In animals, tolerance to the antinociceptive effects of opioids can be observed even after a single dose, and continues to develop over many weeks of drug treatment. A complex interplay of events occurring at the single-cell level and also in neuronal networks is likely to contribute to whole-animal opioid tolerance, with distinct mechanisms being more important at different times during chronic exposure.

The analgesic properties of most of the commonly used opioid drugs occur through activation of the μ-opioid receptor. These are G-protein-coupled receptors and, as with many other G-protein-coupled receptors, can undergo rapid desensitization and internalization after exposure to agonist. The generally accepted mechanism underlying μ-opioid receptor desensitization and internalization begins with phosphorylation of activated receptors by G-protein-coupled receptor kinases (GRKs), followed by arrestin binding. At this point, the receptor is in a desensitized state at the plasma membrane. Arrestin-bound receptors can then be internalized via a clathrin-dependent pathway, and either recycled to the cell surface or downregulated. Opioid receptor desensitization can also be modulated by second messenger-linked protein kinases such as protein kinase C, protein kinase A, nitric oxide, and calcium/calmodulin-dependent kinase.

Despite much investigation, the precise cellular mechanisms underlying opioid tolerance and dependence remain elusive. Because proteomic analysis of identified global proteins can provide expression profiles of proteins and their posttranslational modifications in cells, tissues, and organs, this technique has been used to study tolerance in a rat model. In this study, doses of morphine were repeatedly administered intrathecally to induce tolerance at the spinal cord level in rats. These investigators found that 8 proteins were significantly up-regulated or down-regulated in the spinal cord after morphine tolerance developed, including proteins involved in targeting and trafficking of the glutamate receptors and opioid receptors, proteins involved in oxidative stress, and cytoskeletal proteins, some of which were confirmed by Western blot analysis. It is very likely that these identified proteins may serve as potential molecular targets for prevention of the development of morphine tolerance and physical dependence.

Limitations on Proteomic Research

Two of the key issues encountered by investigators who study the proteome is how much of a particular protein is expressed in a cell or tissue being studied and how to make sense of the enormous amount of protein data generated by every experiment. With each sample of cells or tissue being examined, expression levels of proteins vary tremendously from a few copies to more than a million. Furthermore, the level of protein expressed in a cell or specimen may have little to do with its significance. Essential enzymes and structural proteins are in abundance, whereas vital regulatory proteins are in such tiny quantities that they are almost impossible to detect. Many of these low-level proteins are in multiple forms that govern the targeting, structure, function, and turnover of protein, and it is precisely these proteins that are so crucial to furthering our understanding.
of both health and disease. Finally, at present, our experimental technologies are generating data at a rate and a level of complexity that exceeds our interpretive skills. An average proteomic experiment, for example, might generate 10,000 to 1,000,000 individual data points, with countless potential interactions between data points. Substantial resources are being brought to bear toward the development of software products and international databases for the reliable analysis of computer-generated proteomics data. An integral feature of such programs is the need to establish methods of standardization of data sets across experiments, technology platforms, and physiological/biochemical systems.

GENETIC MECHANISMS OF DISEASE IN CHILDREN

Although individually rare, genetic disorders are collectively common and account for >50% of all admissions to pediatric hospitals. Patients with genetic disorders come to the operating room and procedure areas for all of the usual reasons, in addition to problems related to their genetic disease. However, when compared with the general population, they pose a significantly increased perioperative morbidity and mortality risk because of their anatomic, physiologic, and metabolic abnormalities. The need for the anesthesiologist to anticipate, evaluate, and research the risks posed by these patients is often a daunting task. The next section reviews the demographics and mechanisms of genetic disorders and thereby provides a context for understanding genetic disease.

Traditionally, genetic disorders have been linked to the “one gene–one protein–one disease” hypothesis. However, recent advances in the field of molecular genetics and biotechnology have afforded us the opportunity to greatly expand our knowledge of genetics. We now know that the mechanisms of inherited disorders are often significantly more complex, and consequently much more intriguing, than originally thought.

Classical Mendelian disorders with relatively simple genetic mechanisms do exist, but turn out to be far more rare than originally thought. Three common and well-known examples include Marfan syndrome, sickle cell disease, and achondroplasia. Marfan syndrome occupies a special place in the history of medicine and science owing to the number of seminal discoveries and conceptual breakthroughs that have been associated with this disorder.

A 50-year-long analysis of the clinical and genetic features of Marfan syndrome ultimately led Victor McKusick to delineate it as the founding member of a larger group of congenital conditions that he defined as the heritable disorders of the connective tissue, and which he predicted to be the result of structural or metabolic dysfunctions of extracellular matrix proteins. The demonstration in 1991 that mutations in the fibrillin-1 gene (FBN1) cause Marfan syndrome confirmed McKusick’s prediction, and in addition represented an early successful example of the discovery of a disease-causing gene on the basis of the convergence of genetic linkage studies and the candidate gene approach. Fifteen years later, the unexpected finding that increased transforming growth factor β (TGFβ) signaling is part of the molecular pathogenesis of Fbn1-deficient mice has paved the way to a new drug-based strategy against the life-threatening manifestations of Marfan syndrome and in Loeys–Dietz syndrome, a genetic disease that shares selected features with Marfan syndrome (pectus deformity, scoliosis, and aortic root aneurysm) but can be distinguished on the basis of characteristic features, including hypertelorism, cleft palate, bifid or broad-based uvula, and the absence of ectopia lentis.

Loeys–Dietz syndrome is caused by heterozygous mutations in the genes encoding the TGFβ receptors 1 and 2. These mutations result in increased TGFβ signaling in both cells and involved tissues from affected patients. The arterial disease in these patients is notable for arterial tortuosity in large- and medium-sized vessels (prominently of neck vessels) and the high risk of aneurysms and dissection throughout the arterial tree. Furthermore, aortic root aneurysms tend to dissect at younger ages and smaller dimensions than those seen in Marfan syndrome. One series of 52 families reported an average age at death of 26 years among affected individuals.

Losartan, an angiotensin II type 1 receptor (AT1), antagonizes TGFβ in human and animal models of chronic renal insufficiency and cardiomyopathy and is a Food and Drug Administration–approved medication widely used to decrease arterial blood pressure, a desirable effect in individuals with Marfan syndrome and aortic root aneurysm. Habashi et al. performed a blinded randomized study comparing the efficacy of losartan with propranolol, which is representative of β-blocking agents widely used in patients with Marfan syndrome, and placebo. The doses of β-blockade and losartan were titrated to achieve a comparable blood pressure response. Treatment was started at 7 weeks, after echocardiographic documentation of aortic root dilation. Fibrillin-1–deficient mice treated with propranolol showed a reduction in the aortic root growth rate during a 6-month period in comparison with the placebo mice; however, the growth rate was still significantly greater than in wild-type mice. However, mice treated with losartan could not be distinguished from wild-type mice by any variable, including absolute aortic root size, aortic root growth rate, aortic wall thickness, or histologic architecture.

Another well-known example is sickle cell disease. All patients with sickle cell disease carry the exact same DNA base pair substitution in the exact same location of the β globin gene. This results in an amino acid substitution that changes the shape and the function of the globin molecule in a predictable way. Similarly, all patients with achondroplasia have a single base pair substitution of the FGF3 (fibroblast growth factor receptor-3) gene. This helps to explain the consistency of findings in the achondroplasia phenotype. Interestingly, mutations at other locations in the FGF3 gene are now known to cause other types of skeletal dysplasias. On the other hand, another common inherited disorder, cystic fibrosis, is known to result from changes in the CFTR (cystic fibrosis transmembrane receptor) gene, but >1000 different disease-causing mutations have been reported within this single gene. This results in significant variation in patient phenotype, depending on the effect of the mutation on the resulting protein’s function. Because most commercial labs test for only between 23
and 100 different mutations, interpreting CFTR mutation testing is significantly complicated by the known risk of false negative results. This raises a particular challenge to the prenatal provider, because routine cystic fibrosis carrier screening has become the standard of care in obstetrical practice in this country.

Although single gene or classic Mendelian inheritance is familiar to all physicians, many examples of complex, or non-Mendelian, inheritance are now known to exist. These include disorders of trinucleotide repeats, errors in imprinting, and gene dosage effects. In addition, the “one gene–one protein–one disease hypothesis” must be updated because there are multiple examples in which 1 gene coding for multiple different proteins can result in several varied disorders. Conversely, a single disorder, or group of clinically related disorders, can be caused by mutations in any 1 of several genes.

The following clinical syndromes are presented, with a discussion of what is currently known about the complex, or nontraditional, genetic mechanisms specific to each condition.

**Trinucleotide Repeat Disorders. Example: Fragile X Syndrome**

Trinucleotide repeats are simply 3 DNA nucleotides that are repeated over and over at a certain position within a gene. Typically, the number of repeats is inherited in stable fashion from a parent to a child. However, in certain situations, the number of repeats can expand during meiosis, resulting in an increased number of repeats in the subsequent generation. The larger the number of repeats, the more unstable the stretch of DNA becomes, and the more it is likely to expand with each future generation. When a trinucleotide repeat reaches a critical length, there is a negative effect on gene function or expression, leading to clinical disease. Fragile X syndrome is an X-linked semidominant disorder caused by expansions of the CGG trinucleotide repeat within the fragile X mental retardation (FMR-1) gene. Expansions of >200 repeats are known to turn off FMR-1 gene expression, resulting in mental retardation. Smaller expansions (premutations) are now known to cause a tremor-ataxia syndrome in otherwise normal adults. Premutations are now associated with a change affecting gene splicing; therefore only a small amount of functional protein results. Interestingly, a certain portion of the population carries multiple copies of SMN2 on any given chromosome. As it turns out, in patients with 3 or more copies of the SMN2 gene, the additive effect of the small amount of normal protein made from each gene is enough to mitigate the effects of the SMN1 gene mutations. These patients will likely develop the milder forms of SMA (types II and III).

**Gene Dosage. Example: Spinal Muscular Atrophy**

Spinal muscular atrophy (SMA) is an autosomal recessive disorder causing degeneration and loss of the lower motor neurons and is characterized by progressive muscle weakness. Type I SMA, which represents 70% of all patients presents within the first few months of life, causes death by 1 to 2 years of age. Type II and type III are progressively milder forms of the same disease with later onset and longer lifespans. Originally, it was assumed that different genetic mutations would be responsible for the different subtypes of SMA. However, it is now known that the same mutation in the SMN1 (spinal motor neuron 1) gene causes all 3 types of SMA. This common mutation is a deletion of the part of the gene that codes for the terminal portion of the protein. From a clinical standpoint this means that the standard deletion test alone cannot predict the type of disease or prognosis for a family. Very recently, it has been found that a related gene, SMN2 (spinal motor neuron 2), plays the critical role in determining the severity of SMA. SMN2 lies in tandem to SMN1, and differs from it in only 5 nucleotides. One of those nucleotide differences results in a change affecting gene splicing; therefore only a small amount of functional protein results. Interestingly, a certain portion of the population carries multiple copies of SMN2 on any given chromosome. As it turns out, in patients with 3 or more copies of the SMN2 gene, the additive effect of the small amount of normal protein made from each gene is enough to mitigate the effects of the SMN1 gene mutations. These patients will likely develop the milder forms of SMA (types II and III).

**Imprinting Disorders. Example 1: Prader Willi and Angelman Syndromes; Example 2: Beckwith Wiedemann Syndrome and Silver Russell Syndrome**

Genomic imprinting appears to be a mammal-specific phenomenon whereby differential gene expression according to parent of origin has evolved as a means to regulate many complex pathways related to growth, metabolism, and neurologic development. This means that some of our genes are expressed differently depending on whether we inherited them from our mother or our father. Imprinting is accomplished by an epigenetic mechanism. This would involve, for example, methylation of specific DNA sequences, without changing the underlying genetic code. In most instances, adding methyl groups to DNA nucleotides will halt expression of the gene. This process occurs with very specific timing in relation to development of an embryo and is reset in the sex-specific gametes of each individual. Alterations of these imprinting processes can result in well-defined genetic syndromes, many of which show alterations in growth, metabolism, and neurologic development. A well-characterized example is the interesting story of 2 very different conditions: Prader Willi and Angelman syndromes. Both are most often caused by an identical deletion on the long arm of chromosome 15. However, because this is an imprinted region of the genome, when the chromosome that has undergone the deletion has been passed on from the child’s father, Prader Willi syndrome results. On the other hand, when the deleted chromosome has been passed on from the mother, Angelman syndrome results. More recently, it has been demonstrated that alterations of a single imprinting control region on chromosome 11 can lead to opposite disorders of congenital growth (an overgrowth syndrome known as Beckwith–Wiedemann syndrome and an undergrowth syndrome known as Silver–Russell syndrome). Interestingly, there is now evidence that the use of assisted reproductive techniques such as in vitro fertilization may heighten the susceptibility of embryos to epigenetic deregulation, leading to increased risk for certain imprinting disorders, specifically, Beckwith–Wiedemann syndrome and Angelman syndrome.

**One Gene Causing Multiple Diseases. Example: Progeria and the Laminopathies**

Hutchingson–Gilford progeria syndrome is characterized by profound failure to thrive, typical facies, alopecia, and
some features of accelerated aging, including loss of subcutaneous fat, stiffness of joints, bony changes, and atherosclerosis. Mental and motor development are usually normal. Average lifespan is 13 years, and death is usually secondary to coronary artery or cerebrovascular disease. Virtually all patients with classic progeria have recently been shown to carry the same single nucleotide change in a gene called Lamin A (LMNA). Lamins are filament proteins that form the scaffolding under the nuclear envelope of the cell. Mutations in these proteins likely affect stability of the nuclear envelope, resulting in anomalies of particular tissues including muscle, bone, tendons, and skin. Interestingly, multiple distinctly different conditions are also now known to be caused by mutations in this single gene and include various forms of muscular dystrophy, cardiomyopathy, cardiac conduction defects, lipodystrophies, and peripheral neuropathy (these include Emery–Dreifuss muscular dystrophy, familial dilated cardiomyopathy and conduction system defects, Dunnigan-type familial partial lipodystrophy, limb-girdle muscular dystrophy, Charcot Marie tooth disease 2B1, mandibuloacral dysplasia, and atypical Werner syndrome). There are likely several explanations for the differences in phenotype resulting from changes in a single gene. These include (a) mutations in various regions of the gene, (b) alternative gene splicing that results in several different proteins with separate functions, and (c) differential tissue expression of the various gene products.

Mutations in a Family of Signal Pathway Genes Resulting in a Family of Overlapping Clinical Syndromes. Examples: RASopathies

The RAS (rat sarcoma gene) kinase pathway is a well-studied, highly conserved cascade important in growth factor and cytokine signaling. Output from this pathway will, in part, determine whether cells will proliferate, differentiate, or die. It has long been known that somatic mutations in the RAS pathway are seen in multiple malignancies such as adenocarcinomas of the pancreas, colon, and lung; in tumors of the thyroid and bladder; and in myeloid leukemia. More recently, it was determined that germline (constitutional, as opposed to acquired) mutations in this same pathway lead to abnormalities in embryonic development, resulting in a group of overlapping syndromes characterized by neuro-cardio-facial-cutaneous findings. Interestingly, most, but not all, of these syndromes have tumor predisposition, likely as a result of the same abnormalities in growth signaling seen in cancer patients with the acquired RAS mutations. These syndromes are collectively referred to as RASopathies, reflecting their common origin in mutations in various genes of the RAS pathway.

Noonan syndrome is characterized by typical facial features, congenital heart defects, short stature, webbed neck, and developmental delay of varying degrees. Costello syndrome is characterized by prenatal overgrowth, postnatal failure to thrive, typical facies, congenital heart disease including hypertrophic cardiomyopathy, short stature, mental retardation, deep palmar and plantar creases, nasal and perianal papillomata, and an increased risk for tumors including rhabdomyosarcoma and bladder carcinoma. Cardio-facial-cutaneous syndrome is characterized by congenital heart disease, typical facies, and multiple skin anomalies including hyperkeratosis, ichthyosis, keratosis pilaris, and eczema. All patients have developmental delay. An increased risk for tumors has not been reported. Neurofibromatosis, type 1, is characterized by pigmentary anomalies and a variety of benign and malignant tumors. Patients are at risk for neurologic complications, short stature, and macrocephaly.

Anesthetic Management

It is beyond the scope of this manuscript to discuss the perioperative evaluation and intraoperative care of these and other patients with genetic disease. Although many textbooks and on-line sources are available to guide the anesthesiologist, the authors believe that the on-line Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/Omim) is the most comprehensive database available and should be the starting point in any search. In addition the National Library of Medicine also supports a site entitled Gene Reviews (http://www.ncbi.nlm.nih.gov/sites/GeneTests), which provides comprehensive and up-to-date clinical summaries on most common single-gene disorders. When combined with search engines such as Google and the Web sites of virtually every rare and not-so-rare genetic disease, the anesthesiologist can rapidly obtain invaluable information about the perioperative care of these patients.

Summary

A fundamental understanding of all biological processes requires an understanding of the molecular basis of cellular function. The discipline of molecular biology focuses on the genetic information in cells: how is the inherited information encoded within DNA and how is this information regulated and expressed so that cells of a multicellular organism develop from a single cell to highly specialized cells in a complex and integrated organism? For the practicing anesthesiologist, an understanding of the molecular mechanisms by which a differentiated cell develops and maintains its specialized functions is critical to a more in-depth understanding of human health and disease. We have provided a primer on congenital malformations, pharmacogenetics, and proteomics to help provide the clinician anesthesiologist with the basic knowledge needed to understand the potential implications of inherited patterns on perioperative anesthetic planning and risk.

REFERENCES


