

David C. Warltier, M.D., Ph.D., Editor

Anesthesiology 2005; 102:663-71

© 2005 American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins, Inc.

Pharmacogenetics of Anesthetic and Analgesic Agents

Stephen N. Palmer, Ph.D.,* N. Martin Giesecke, M.D.,† Simon C. Body, M.B., Ch.B., M.P.H.,†
Stanton K. Shernan, M.D.,† Amanda A. Fox, M.D.,‡ Charles D. Collard, M.D.§

Predicting a patient's response to a particular drug has long been a goal of clinicians. Rapid advances in molecular biology have enabled researchers to identify associations between an individual's genetic profile and drug response. Pharmacogenetics is the study of the molecular mechanisms that underlie individual differences in drug metabolism, efficacy, and side effects. The pharmacogenetics of commonly used anesthetic and analgesic agents are reviewed.

PREDICTING a patient's response to a particular drug has long been a goal of clinicians. *A priori* knowledge of which drug and dosage would be most effective for an individual patient, as well as which drugs might provoke idiosyncratic adverse reactions, would greatly facilitate medical care. The significance of such knowledge is highlighted by a meta-analysis of US hospitals from 1966 to 1996 in which a 6.7% incidence of serious adverse drug reactions was reported (*i.e.*, drug reactions associated with prolonged hospital stay, death, or permanent disability but not including adverse reactions caused by drug administration errors or overdose).¹ Adverse drug reactions typically lengthened hospital stays by 1-4 days and increased costs by \$2,300-5,600.² Therefore, the ability to predict a patient's response to a particular drug is of both medical and socioeconomic importance.

Rapid advances in molecular biology have enabled researchers to identify associations between an individual's genetic profile and drug response. *Pharmacogenetics* refers to the study of inherited differences (variation)

in drug metabolism and response. In contrast, *pharmacogenomics* refers to the study of the many different genes that determine drug behavior. The purpose of this review is to describe the pharmacogenetics of commonly used anesthetic and analgesic agents (table 1). Note that this review focuses only on drugs directly related to the provision of anesthesia. Individuals interested in the pharmacogenetics of other drugs commonly used in the perioperative or intensive care unit setting (*e.g.*, β blockers, antiarrhythmics) are referred to the excellent reviews by Bukaveckas *et al.*,³ Body and Shernan,⁴ and Iohom *et al.*⁵

Basic Molecular Concepts

The molecular structure of every protein present in humans is encoded by DNA. DNA consists of four types of nucleotides, each containing a phosphate group, a sugar, and one of four purine or pyrimidine bases: adenine (A), guanine (G), thymine (T), or cytosine (C). DNA exists as a double helix within the cell nucleus, with base pairing of purines (A and G) to pyrimidines (T and C) between the two backbone strands of phosphate and sugar residues. Variation within the sequence of these base pairs comprises the genetic code.

Each DNA strand serves as a template for messenger RNA (mRNA) transcription catalyzed by RNA polymerase within the cell nucleus. A promoter region, typically 25-200 base pairs proximal to the 5' transcription initiation site, determines which of the two DNA strands will be transcribed into mRNA by orientating RNA polymerase in a specific direction. The amount and timing of DNA transcription is also regulated by binding of various gene regulatory proteins known as *transcription factors* to the promoter region or to specific DNA sequences distant from the promoter region known as *enhancer* regions. Promoter and enhancer regions together thus modulate protein production by regulating the amount of mRNA transcribed. After transcription, the mRNA is further modified within the cell nucleus *via* splicing whereby introns (areas within the gene that do not code for protein) are excised by enzymes called *spliceosomes*. The remaining exons (areas of DNA that code for protein) are then joined together. The final mature mRNA is

This article is accompanied by an Editorial View. Please see: Allen PD: Anesthesia and the human genome project: The quest for accurate prediction of drug responses. ANESTHESIOLOGY 2005; 102:494-5.

* Scientific Medical Writer, † Assistant Professor of Anesthesia, ‡ Instructor of Anesthesia, § Associate Professor of Anesthesia.

Received from the Department of Cardiovascular Anesthesia, Texas Heart Institute, St. Luke's Episcopal Hospital, Houston, Texas, and the Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts. Submitted for publication July 21, 2004. Accepted for publication October 26, 2004. Supported by the National Heart, Lung and Blood Institute (HL-068774; SCB), Bethesda, Maryland.

Address reprint requests to Dr. Collard: Department of Cardiovascular Anesthesia, Texas Heart Institute, St. Luke's Episcopal Hospital, 6720 Bertner Avenue, Room 0520, MC1-226, Houston, Texas 77030. Address electronic mail to: ccollard@heart.thi.tmc.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

Table 1. Example Genotypes Linked to Individual Differences in the Metabolism, Efficacy, and Side Effects of Commonly Used Anesthetic Agents

Drug	Gene	Allele	Effect Associated with Allele*
Halothane, other inhalation anesthetics	CYP2E1	Unknown	Hepatitis ³⁰⁻³²
Halothane, isoflurane, succinylcholine	RYR1	≥23 different mutant alleles	Malignant hyperthermia syndrome ^{17,19-24}
Succinylcholine, mivacurium	Plasma butyrylcholinesterase	"Atypical" allele "Silent" allele	Prolonged muscle relaxation ^{8,10}
Diazepam	CYP2C19	G681A	Increased half-life, prolonged sedation, greater likelihood of unconsciousness ¹¹⁻¹³
Midazolam	CYP3A4	A290G (*1B)	Reduced clearance of systemic (but not oral) midazolam ¹⁶
Midazolam	CYP3A5	A22893G (*3)	Reduced clearance of systemic (but not oral) midazolam ^{14,15}
Morphine, morphine-6-glucuronide	μ-Opioid receptor	A118G	Reduced nausea, vomiting, sedation, drowsiness ^{40,41}
Morphine	Uridine diphosphate glycosyl transferase	C-161T and C802T	More rapid glucuronidation ⁴³
Codeine	CYP2D6	G1846A (*4) Deletion of gene (*5) 1707T>del (*6)	Slower conversion of codeine to morphine; reduction in analgesic effect (but not adverse effects) ⁴⁶
Tramadol	CYP2D6	G1846A (*4) Deletion of gene (*5) 1707T>del (*6)	Slower conversion to O- and N-demethyltramadol; greater need for rescue analgesics after surgery ^{49,50}
Methadone	CYP2D6	G1846A (*4) 1707T>del (*6)	Slower metabolic clearance (although not consistently) ^{51,52}
Celecoxib, naproxen, piroxicam, ibuprofen, flurbiprofen	CYP2C9	A1075C (*3)	Slower metabolism ⁶⁴⁻⁶⁹
Acetaminophen	CYP2E1	c2	Accelerated elimination rate ⁷³
Acetaminophen	Tumor necrosis factor β	B2	Lower risk of encephalopathy in patients with acetaminophen-induced acute liver failure ⁷⁴
Various NSAIDs	HLA-DRB1	*11	Higher risk of anaphylactoid reaction ⁷⁵

* Effects described are those of the variant allele compared with the wild-type allele for each polymorphism.

CYP = cytochrome P450; HLA = human leukocyte antigen; NSAID = nonsteroidal antiinflammatory drug; RYR1 = ryanodine receptor.

transported to the cell cytoplasm where it undergoes ribosomal translation into the various proteins of the body. Each sequential triplet of mRNA bases is called a *codon*, with each codon encoding an amino acid.

A *gene* is a hereditary coding unit composed of a specific DNA sequence occupying a specific position or locus within a chromosome (*i.e.*, a long DNA molecule and its associated proteins). Humans have 23 pairs of chromosomes. All genes have a common structure that includes a 5' untranslated region, exons, introns, and a 3' untranslated region. An *allele* is any of two or more alternative forms of a gene occupying the same chromosomal locus. The most common type of human genetic or allelic variation is the single-nucleotide polymorphism (SNP), a "point mutation" or position at which two alternative nucleotides occur. To date, more than 13 million SNPs have been identified. Allelic variation may also occur secondary to other types of mutations, including the insertion, deletion, translocation, or inversion of DNA segments. The words *mutation* and *polymorphism* can be used interchangeably, but in general, *mutation* refers to a variation that occurs in less than 1% of

the population, and *polymorphism* refers to a variation that occurs in more than 1% of the population.

Although most mutations are "silent" (*i.e.*, they have no effect on the composition or amount of resultant protein produced), allelic variation may significantly alter the phenotype of an organism (*i.e.*, its outward, physical manifestations). For example, allelic variation may significantly affect drug metabolism, efficacy, and side effects (fig. 1). Further, because every individual carries two alleles of most genes (one inherited from each parent), the clinical effects of a mutation may be magnified when the mutation occurs in both alleles (homozygous expression), rather than in a single allele (heterozygous expression). An individual who expresses an abnormal, dysfunctional copy of a drug-metabolizing enzyme on only one chromosome (heterozygote) may have a clinically insignificant reduction in drug clearance. However, if the same individual were to express abnormal copies of the enzyme on both chromosomes (homozygote), then a clinically significant reduction in drug metabolism is much more likely to occur because of production of a mutated enzyme with little or no

Silent Mutation (Same Amino Acid)

Normal CCT CAT TGG AGT GAT GGT TTT CGT GAG
Pro His Trp Arg Asp Gly Phe Arg Glu

Mutant CCT CAT TGG AGT GAT GGC TTT CGT GAG
Pro His Trp Arg Asp **Gly** Phe Arg Glu

Conservative Mutation (Structurally Similar Amino Acid)

Normal CCT CAT TGG AGT GAT GGT TTT CGT GAG
Pro His Trp Arg Asp Gly Phe Arg Glu

Mutant CCT CAT TGG AGT GAT GGT TTA CGT GAG
Pro His Trp Arg Asp Gly **Leu** Arg Glu

Functional Coding Mutation (Altered Protein Structure or Function)

Normal CCT CAT TGG AGT GAT GGT TTT CGT GAG
Pro His Trp Arg Asp Gly Phe Arg Glu

Mutant CCT CAT TGG AGT GAA GGT TTT CGT GAG
Pro His Trp Arg **Glu** Gly Phe Arg Glu

Fig. 1. A single-nucleotide polymorphism within a gene may have no effect on the resultant structure or function of a protein because the altered codon encodes an amino acid that is the same as (silent mutation) or structurally similar to (conservative mutation) the amino acid encoded by the original codon. Alternatively, a single-nucleotide polymorphism may significantly alter protein structure or activity (coding mutation). From Body and Shernan⁴; used with permission.

activity. Therefore, the effects of a mutation may be additive. Some mutations act in a dominant fashion, so that expression of a single abnormal gene copy is sufficient to result in disease. In contrast, other mutations are recessive in that abnormal gene copies on both chromosomes must be expressed for disease to occur.

Cystic fibrosis or sickle cell disease are examples of Mendelian traits, traits for which single or multiple changes in the genetic code induce a profound response in a “yes/no” fashion (*i.e.*, an individual either has or does not have the trait). Mendelian traits or diseases are relatively unaffected by nongenetic or environmental factors (*e.g.*, diet, amount of exercise, carcinogen exposure). In contrast, the variability in postoperative analgesic requirements among individuals is an example of a “complex” trait in which many genetic and environmental factors interact to influence drug metabolism, efficacy, and side effects. Examples of environmental factors that may affect drug pharmacokinetics and pharmacodynamics include previous drug or alcohol use, recent ingestion of a meal, and the method of drug preparation and administration. Further, the results of genetic testing in patients with Mendelian and complex traits must be considered in the context of mutation variability, gene penetrance, and gene expressivity. *Mutation variability* (heterogeneity) refers to the fact that

many different mutations can cause the same disease or trait and that these mutations can be in the same or different genes. Most genetic tests screen for only the most common mutations. *Gene penetrance* refers to the probability of getting a disease or expressing a specific trait when a mutation is present. In the situation of incomplete gene penetrance, an individual with a mutation may never manifest the disease. Finally, *gene expressivity* refers to the range of disease severity for a mutation. Even patients with Mendelian diseases may display considerable heterogeneity with regard to disease severity, suggesting that in essence there are no “simple” diseases and that all diseases are complex.

Polymorphism Nomenclature

Gene polymorphisms are labeled using three different nomenclature systems. The first system uses a number to signify the gene locus where a single nucleotide substitution occurs. The letter before the number signifies the nucleotide most commonly found at the gene locus (*i.e.*, the “wild-type” or major allele), whereas the letter after the number represents the nucleotide found in the mutant or minor allele. Therefore, the A118G SNP of the μ -opioid receptor gene codes for the replacement of the nucleotide adenine (A) at base pair 118 with guanine (G). Alternatively, this polymorphism may be written as 118 A/G or 118 A>G. Coding polymorphisms that affect a single gene codon and result in an amino acid switch may also be labeled using the three-letter abbreviations of the exchanged amino acids. Therefore, the Asp70Gly polymorphism of the butyrylcholinesterase gene involves a glycine substitution for aspartate at the 70th amino acid of the protein. A third commonly used polymorphism nomenclature system involves numbering the different alleles (*e.g.*, *1). For example, the CYP2D6*5 allele is the fourth identified variant in the cytochrome P-450 (CYP) enzyme 2D6. This system is the least descriptive but most flexible because it can accommodate single or multiple concordant mutations of any degree of complexity, including insertions or deletions that alter huge sections of the gene. Finally, it should be noted that numerous polymorphisms are still known by confusing, nonstandard historic abbreviations.

Pharmacogenetic Approaches

Pharmacogenetic studies can be loosely categorized into two methodologic approaches: linkage studies and gene association studies. *Genetic linkage* is the phenomenon whereby alleles at loci close together on the same chromosome tend to be inherited together, because it is rare for crossover to occur between the loci during meiosis (*i.e.*, chromosomal halving). A *haplotype* is a combination of alleles at closely linked loci on a single chromosome that tend to be inherited together. SNPs that are linked together are said to be in *linkage disequilibrium* because they are not randomly inherited and

therefore have not reached equilibrium within a population. Linkage can be used to map disease genes by typing polymorphic DNA markers to see if their alleles cosegregate with disease among related subjects. If relatives share marker alleles more often than would be expected by chance, this suggests that a susceptibility locus may be linked to the marker. Linkage studies typically examine families with a high prevalence of an anomalous drug response (e.g., a rare adverse reaction), comparing thousands of genes between family members with and without the anomaly. In this manner, the point of chromosomal swapping in the genome most significantly related to the anomaly can be determined. However, the accuracy of this technique is typically limited to a large area within a single chromosome, necessitating further studies to narrow the region to a specific gene. Linkage studies were more commonly used in the past, when the small number of known gene polymorphisms made it necessary to study related individuals to reduce potential interindividual genetic variability.

Rapid advances in molecular biology and the Human Genome Project have led to the identification of millions of novel polymorphisms in recent years. Further, our understanding of the enzymes, receptors, and other elements involved in drug pharmacokinetics and pharmacodynamics has also greatly improved, enabling identification of novel candidate genes. An increasing number of studies have thus focused on disease expression or the occurrence of adverse clinical outcomes in individuals expressing a specific genotype. Gene association studies are used to determine the influence of human genetic variation on the pathogenesis of disease, variability in disease expression, and response to treatment. SNPs in particular have been shown to be useful as genetic markers for identifying disease genes by association analysis of patients and controls. Most current pharmacogenetic studies therefore use the gene association approach because such analyses rely on fundamental case-control study methodology, are statistically more powerful than linkage studies, and do not require the study of family members.

Although an extremely powerful research tool, the gene association study is not without limitations.⁶ First, the primary endpoints of a gene association study must be sufficiently powered to account for genetic admixture within the study population (i.e., the inclusion of patients originating from many distinct genetic backgrounds). This is especially true for complex diseases with significant heterogeneity (i.e., diseases with multiple genetic origins). Association analyses that rely on the assumption that trait-influencing genes are inherited by descent may not identify influential genes, because the association will be divided between multiple loci in the sample of affected individuals. Therefore, negative findings in an association study examining only several hundred patients of high genetic admixture should be inter-

preted with caution. Second, it is crucial for data interpretation that the appropriate statistical analyses are applied, preferably by a statistician experienced in genetic research. Gene association studies typically involve multiple comparisons of many different continuous and noncontinuous variables within different populations. Special care must therefore be taken to avoid identification of spurious gene associations. Moreover, identification of a positive association between a specific genotype and clinical outcome does not necessarily imply causality. The identified genotype may actually be clinically silent, but linked to one or more other genotypes that individually or collectively form a disease haplotype. Along these lines, several investigators have advocated for building a "haplotype map" of the human genome that will make it easier, faster, and perhaps cheaper to find disease-causing or disease-predisposing genes. Instead of searching through a giant haystack of millions of SNPs, scientists would be searching through bundles of 10,000 to 50,000 bases each. Haplotype mapping may also greatly increase the sensitivity and specificity of predicting how genotypic variation will affect specific clinical outcomes.⁷

Neuromuscular Blocking Agents

The effectiveness of neuromuscular blockers such as succinylcholine and mivacurium is strongly associated with genetic factors. Variations in the gene for plasma butyrylcholinesterase (pseudocholinesterase), the enzyme that hydrolyzes these drugs, have been correlated with dramatic interindividual differences in the duration of drug-induced muscular paralysis. Normally, patients completely recover neuromuscular function within 5–10 min after receiving 1.0–1.5 mg/kg succinylcholine. However, heterozygous (single allele) expression of the butyrylcholinesterase Asp70Gly polymorphism results in production of a less effective form of plasma butyrylcholinesterase. These patients typically take three to eight times longer to recover neuromuscular function after succinylcholine administration. Homozygous expression (both alleles) of the butyrylcholinesterase Asp70Gly polymorphism prolongs the recovery of neuromuscular function even further, resulting in a recovery period up to 60 times longer than that associated with the normal allele.⁸ Allelic variation within the butyrylcholinesterase gene has also been shown to significantly prolong mivacurium-induced muscle paralysis.⁹ Therefore, it is not surprising that patients who require prolonged mechanical ventilation for the treatment of prolonged neuromuscular blockade after mivacurium or succinylcholine administration are more likely to carry atypical butyrylcholinesterase alleles.¹⁰ Avoidance of these drugs may be prudent in patients with *a priori* knowledge of an abnormal butyrylcholinesterase genotype to reduce recovery time and cost after surgery.

Benzodiazepines

Most benzodiazepines are metabolized by hepatic cytochrome P-450 enzymes to more polar metabolites excreted in the bile or urine. The half-life of diazepam in individuals homozygous for the A allele of the cytochrome CYP2C19 G681A polymorphism is four times longer than individuals homozygous for the major (G) allele, presumably because of markedly decreased CYP2C19 metabolic activity.¹¹ Individuals heterozygous for the A allele have diazepam half-lives somewhere between these two extremes.^{11,12} These genetic differences may manifest clinically as prolonged sedation or unconsciousness after diazepam administration.¹³ In contrast to diazepam, the clinical response to midazolam thus far has been only modestly associated with genetic factors. Although polymorphisms within the cytochrome CYP3A4 and CYP3A5 genes have been shown to be associated with reduced midazolam clearance, the clinical significance of these polymorphisms is relatively small because of alternative means of excretion and metabolism.¹⁴⁻¹⁶

Inhalation Anesthetics

To date, the bulk of pharmacogenetic research on inhaled anesthetics has focused on finding the genetic causes of adverse reactions to these drugs, with the best understood example being malignant hyperthermia syndrome (MHS). Triggered by volatile anesthetic inhalation or intravenous succinylcholine administration, MHS is a hypermetabolic disorder of skeletal muscle that is often, but not always, associated with significant increases in body temperature to 43.3°C (110.0°F) or higher. Approximately 1 in 15,000 children and 1 in 50,000 adults are susceptible to MHS. However, the reaction does not always occur in susceptible individuals, so the actual prevalence of MHS may be higher.¹⁷ It is intriguing to note the much higher incidence in children than in adults, perhaps indicating that MHS behaves like a complex genetic disease. Pharmacogenetic studies of MHS have found numerous associations with variations in the ryanodine receptor (RYR1) gene, with approximately 50% of cases involving mutations within this gene. In addition, a mutation in the α_1 subunit of the voltage-dependent calcium channel has been associated with 1% of North American MHS cases.^{17,18} However, the true percentage is difficult to determine because at least 23 different RYR1 polymorphisms seem to be associated with MHS.¹⁹ Particularly severe cases of MHS tend to occur in individuals with central core disease, a muscular disorder also known to be associated with RYR1 polymorphisms.^{20,21}

The diversity of RYR1 polymorphisms associated with MHS, as well as the fact that many MHS-susceptible individuals have no known RYR1 polymorphisms, makes genetic testing for this disorder impractical at the current time. Further compounding the problem are ethnic

variations in the frequency of MHS-related polymorphisms. Studies examining RYR1 polymorphisms have found these polymorphisms in 26, 41, and 70% of American, Italian, and German MHS patients, respectively.²²⁻²⁴ Studies of individuals related to known MHS carriers, but who themselves have not experienced an episode of MHS, typically use the *in vitro* contracture test to determine the presence or absence of the disorder. This test involves extracting muscle cells and exposing them *in vitro* to halothane, caffeine, or other substances which cause muscle cells to contract. Cells extracted from MHS patients typically show a higher-than-normal degree of contraction. Although *in vitro* contracture test results seem to be positively influenced by the presence of MHS-related RYR1 alleles, cells expressing different alleles may respond differently to testing.^{17,25} This variation increases further when different drugs are used to elicit the response, because muscle cells carrying certain RYR1 alleles respond differently to halothane than they do to caffeine.²⁶ Therefore, the degree of association between a particular RYR1 polymorphism and MHS may vary between studies if different test substances are used.

In contrast to MHS, halothane-induced hepatitis seems to result from an immune response to halothane metabolites processed by the cytochrome enzyme CYP2E1.^{27,28} Halothane-induced hepatitis occurs in approximately 1 in 10,000 patients.²⁹ Although its tendency to cluster within families suggests that it is hereditary, the genetic mechanisms involved are not entirely clear, because CYP2E1 activity depends as much on body mass, diet, alcohol consumption, and age as it does on any polymorphisms discovered to date.³⁰⁻³²

The large volume of pharmacogenetic research on inhalation anesthetic adverse reactions sharply contrasts with the virtual absence of literature on the genetic influences on inhalation anesthetic effect. The lack of efficacy data is in part due to the very small observed interpersonal variability in inhalation anesthetic effect and our incomplete understanding of the molecular mechanisms by which inhalation anesthetics work. In addition, there are few findings in the animal literature to spur human research on this topic. Responses to inhalation anesthetics known to be associated with genetic mutations in nonmammalian species often occur only with anesthetic concentrations much higher than those used in mammals.^{33,34} Although mice with a melanocortin-1 receptor mutation were recently shown to have a higher minimum alveolar concentration than control mice not expressing the mutation, almost no other specific efficacy-related polymorphisms have been identified in genetic screening studies using murine or other mammalian models.^{35,36}

Recently, Selzer *et al.* reported the unexpected neurologic deterioration and death of an infant boy who had been anesthetized twice within a short time with nitrous

oxide.^{37,38} Postmortem studies of his cultured fibroblasts established a diagnosis of 5,10-methylenetetrahydrofolate reductase deficiency, an inherited defect in folate metabolism. Analysis of this patient's DNA revealed a complex combination of mutations in his 5,10-methylenetetrahydrofolate reductase gene, including two common mutations (C677T and A1298C) associated with a reduction in 5,10-methylenetetrahydrofolate reductase activity.^{37,38} Nitrous oxide irreversibly oxidizes the cobalt atom of vitamin B₁₂, thereby inhibiting the activity of the cobalamin-dependent enzyme methionine synthase. Methionine synthase catalyzes the remethylation of 5-methyltetrahydrofolate and homocysteine to tetrahydrofolate and methionine. Methionine, by way of its activated form, S-adenosylmethionine, is the principal substrate for methylation in many biochemical reactions, including assembly of the myelin sheath, methyl substitutions in neurotransmitters, and DNA synthesis in rapidly proliferating tissues. The findings and course of events in this patient suggest that limitations in 5-methyltetrahydrofolate synthesis caused by his complex 5,10-methylenetetrahydrofolate reductase mutations, along with partial or complete inactivation of methionine synthase by nitrous oxide, resulted in an extreme deficiency of methionine in the brain and, ultimately death. This case report and subsequent DNA evaluation highlight how underlying genetic influences may unexpectedly and unfavorably alter anesthetic outcomes.

Opioids

The μ -opioid receptor is the principal site of action for clinically used opioids. Two common variants of the μ -opioid receptor are present in 5–10% of Caucasians; A118G and the promotor polymorphism, G-172T.³⁹ Although the G118 allele has no apparent relation to patients' analgesic response to morphine or its active metabolite, morphine-6-glucuronide, preliminary data suggest that the G allele is associated with reduced severity of the adverse effects of the drugs, including nausea, vomiting, pupil dilation, and sedation.^{40,41} Therefore, carriers of the G118 allele may tolerate higher opiate doses than noncarriers do.

Although there seems to be little important genetic variability in the chemical binding of the μ -opioid receptor to agonists, large interindividual differences in post-surgical opioid requirements are frequently observed.⁴² The underlying molecular mechanisms of this phenomenon have yet to be fully elucidated, but it has been suggested that these observations may be explained in part by promoter polymorphisms associated with wide variation in receptor numbers rather than function and the fact that many genes and environmental factors are involved in pain perception. In addition, several polymorphisms have been linked to altered opiate metabolism. For example, uridine diphosphate glycosyl transferase is the enzyme responsible for the 3- and

6-glucuronidation of morphine. Surgical patients homozygous for the C-161T and C802T uridine diphosphate glycosyl transferase gene polymorphisms (which are always inherited together) demonstrate more rapid morphine glucuronidation than heterozygous or wild-type patients.⁴³

Another enzyme important for opioid metabolism is the liver P-450 cytochrome, CYP2D6. CYP2D6 is involved in the processing of approximately 25% of all currently administered drugs, including the conversion of codeine into its active metabolite morphine, the mechanism of codeine-induced analgesia.⁴⁴ CYP2D6 genotypes are highly variable among individuals, not only because there are many different functional and non-functional gene polymorphisms, but also because a "gene duplication" event causes some individuals have more than two copies of the CYP2D6 gene. This results in CYP2D6 production above the "normal" levels seen with the wild-type CYP2D6*1 allele. The prevalence of CYP2D6 gene duplication varies widely by ethnicity, ranging from 0.5% in China to 29% in Ethiopia and averaging 4–5% in the United States.⁴⁵ Patients who rapidly convert codeine into morphine usually have at least one wild-type CYP2D6 allele, known as CYP2D6*1. In contrast, poor metabolizers of codeine (8% of whites and higher frequencies in other ethnic groups) tend to express combinations of the *4, *5, and *6 alleles and are virtually immune to the analgesic effects of codeine.^{46,47} Interestingly, the severity of the adverse effects of codeine does not seem to be associated with the CYP2D6 genotype, thus explaining how codeine can impair driving ability even when the driver's blood does not contain detectable concentrations of morphine.^{46,48}

CYP2D6 genotype is associated with patients' response to other opiates in addition to codeine. An examination of 33 deceased tramadol users at autopsy showed that the number of functional CYP2D6 alleles expressed was strongly correlated with the ratio of tramadol to O- and N-demethyltramadol blood concentrations, suggesting that the rate of tramadol metabolism is influenced by CYP2D6 polymorphisms.⁴⁹ The clinical importance of this finding is highlighted by another study in which abdominal surgical patients with one or more CYP2D6*1 alleles were found to be twice as likely to require additional postoperative analgesics because of increased tramadol metabolism.⁵⁰ CYP2D6 genotype is also associated with the rate of methadone metabolism.⁵¹ However, this relation is not consistent because many individuals with functional CYP2D6 alleles are nonetheless poor metabolizers of methadone.⁵² This may be because methadone metabolism is dependent on other cytochromes in addition to CYP2D6, especially CYP3A4.^{53,54} CYP3A4 has been shown to be important in the metabolism of a number of other opiates, including fentanyl, alfentanil, and sufentanil.^{55,56} Further, it is

interesting to note that CYP3A4 metabolism is 40% more rapid in females than in males. Such sex differences may help to explain why females have a threefold higher incidence of awareness and awaken more rapidly from total intravenous anesthesia.⁵⁷

Recently, the catechol *O*-methyl transferase (COMT) gene was related to the perception of pain.⁵⁸ COMT is an important mediator of catecholamine metabolism and is a modulator of adrenergic and dopaminergic pathways, including those involved in pain transmission. The Val158Met COMT gene polymorphism has been previously shown to be associated with a threefold to fourfold reduction in enzyme activity.⁵⁹⁻⁶¹ Using a human pain model, Zubieta *et al.*⁵⁸ injected the temporomandibular joint with hypertonic saline. The volume of hypertonic saline necessary to reach and maintain a preset level of pain intensity paralleled COMT activity, being lowest in Met homozygotes, intermediate in heterozygotes, and highest in Val homozygotes. Similarly, affective responses to pain were highest in individuals with the lowest COMT activity (Met homozygotes), followed by heterozygotes and Val homozygotes. To track opioid receptor binding, radiolabeled carfentanil was administered and tracked by positron emission tomography. Met homozygotes showed diminished regional μ -opioid system responses to pain (*i.e.*, decreased carfentanil binding) compared with heterozygotes. These data thus demonstrate how allelic variation may alter a drug's efficacy by altering receptor binding and function. Although the subject is beyond the scope of the present article, we refer readers interested in the effects of genetic variation on chronic opiate addiction to the excellent reviews by LaForge *et al.*⁶² and Kreek *et al.*⁶³

Nonsteroidal Antiinflammatory Drugs

Many nonsteroidal antiinflammatory drugs (NSAIDs) are metabolized by the cytochrome enzyme CYP2C9. Individuals with the CYP2C9*3 allele metabolize celecoxib, naproxen, piroxicam, ibuprofen, and flurbiprofen more slowly than those without this allele.⁶⁴⁻⁶⁹ Furthermore, this reduction in metabolism tends to be much larger in *3 homozygotes than in heterozygotes. These findings suggest that *3 carriers may experience more clinical benefit or adverse effects from NSAIDs or both. However, no studies have yet confirmed this.

In contrast, *in vivo* metabolism of diclofenac seems largely unrelated to CYP2C9 genotype.^{65,70,71} Although the CYP2C9*2 and *3 polymorphisms were once thought to be associated with diclofenac-induced hepatotoxicity, no differences have been found in the ability of liver microsomes to metabolize diclofenac in CYP2C9*2 and *3 heterozygotes and CYP2C9*1 homozygotes.⁷² Tracy *et al.*⁶⁶ have speculated that CYP2C9 may bind to diclofenac differently than it does to other NSAIDs in terms of its orientation or binding site, possibly in ways unaffected by the known polymorphisms of this gene.

Genes other than CYP2C9 may also be associated with NSAID metabolism, but the evidence for these associations is sparse. The results of one study suggest that individuals who are homozygous for the c2 allele (which lacks a DNA segment that limits protein manufacture in the wild-type c1 allele) of the cytochrome CYP2E1 metabolize acetaminophen at twice the rate of heterozygous or wild-type individuals.⁷³ Other data imply that a mutant allele (B2) of the tumor necrosis factor β gene protects against encephalopathy in patients with acetaminophen-induced acute liver failure.⁷⁴ Finally, there is preliminary evidence that the *11 allele of the gene for the human leukocyte antigen HLA-DRB1, which is involved in the immune response, may be associated with anaphylactoid reactions to various NSAIDs.⁷⁵ However, more research is necessary to determine the accuracy and clinical relevance of these findings.

Interestingly, no study has yet revealed any association between NSAID activity and cyclooxygenase-2 gene polymorphisms. This is surprising, given that cyclooxygenase-2 inhibition is the mechanism of action for all NSAIDs. It has been suggested that the cyclooxygenase-2 enzyme is so important for maintaining body homeostasis that any polymorphisms that might significantly alter its function have been suppressed by natural selection.⁷⁶ However, this explanation seems unlikely because the G-765C polymorphism of the cyclooxygenase-2 gene was recently shown to be independently associated with a decreased risk of myocardial infarction and stroke.⁷⁷

Conclusions

For decades, anesthesiologists have been aware of individual differences in response to pharmacologic agents, perhaps more so than medical professionals in any other specialty. Common clinical examples from anesthetic practice include prolonged muscle relaxation in response to succinylcholine, the induction of MHS by volatile anesthetics, and the commonly observed wide variation among individuals for postoperative analgesic requirements. Growing pharmacogenetic data make it evident that these and other drug-related phenomena have an underlying genetic component and that genetic variation may significantly affect drug absorption, distribution, metabolism, excretion, and toxicity. Genetic evaluation might shed light on many clinical situations without explanation that are currently "brushed under the carpet," such as surgical patients who do much better or worse than expected or families with suspicious anesthetic histories (*e.g.*, slow awakening or awareness). Further, as we acquire more knowledge about alleles related to drug activity, we may even be able to identify polymorphisms that primarily affect pharmacokinetic events (same drug dose, different serum concentrations) from those that primarily affect pharma-

codynamic events (same drug dose, same serum concentrations, and different drug effects). The study of pharmacogenetics may eventually lead to genetic “fingerprinting” or screening tests of alleles known to significantly affect drug metabolism, efficacy, and side effects. However, the practical applicability of such pharmacogenetic knowledge will depend largely on the cost-benefit ratio associated with performing genetic testing. For example, the medical and economic consequences of halothane-induced MHS are severe. However, if, as mentioned previously, the frequency of MHS susceptibility in adults is 1 in 50,000 and if approximately half of these individuals have mutant alleles of RYR1, MHS-related RYR1 alleles would be expected to be found in 1 in 100,000 patients.¹⁷ Assuming a commercial cost of only \$100 per test (note that current commercial costs are actually considerably higher), universal preoperative RYR1 testing would therefore cost \$10 million to prevent an MHS episode in one adult surgical patient. However, if the testing were restricted to patients with first-degree relatives known to have had an episode of MHS, 1 in 4 of the tested patients would be found to have an MHS-related mutation, thereby preventing one occurrence of MHS for every \$400 spent. Admittedly, this estimate assumes perfect concordance between a positive test for MHS-related RYR1 alleles and actual MHS susceptibility. Nonetheless, genotyping costs are rapidly decreasing, with actual costs being only pennies per allele. High-throughput screening may thus soon become a reality, especially if clinicians also do their part by becoming sophisticated in genetic issues (e.g., the risks of DNA cross-contamination in genotyping, difficulties in high-fidelity heterozygote detection, interpretation of genetic test results), and by demanding that such tests reach the marketplace.

There are additional questions that must be answered before pharmacogenetics becomes a routine part of laboratory medicine. When should genetic testing be performed: before a patient goes on medication or after there is therapeutic failure? How should dosing be adjusted? Should alternative therapy be considered based on a patient's genetic makeup? Therefore, although pharmacogenetics has the potential to increase drug efficacy, prevent drug toxicity, eliminate costly and ineffective drug alternatives, and decrease physician follow-up visits, there remains a strong need for prospective, sufficiently powered gene association studies conducted in well-defined, highly phenotyped populations. Only then can we begin to critically evaluate the relative importance or clinical significance of various gene associations.

References

- Lazarou J, Pomeranz BH, Corey PN: Incidence of adverse drug reactions in hospitalized patients: A meta-analysis of prospective studies. *JAMA* 1998; 279: 1200-5
- Rodriguez-Monguio R, Otero MJ, Rovira J: Assessing the economic impact of adverse drug effects. *Pharmacoeconomics* 2003; 21:623-50
- Bukaveckas BL, Valdes R Jr, Linder MW: Pharmacogenetics as related to the practice of cardiothoracic and vascular anesthesia. *J Cardiothorac Vasc Anesth* 2004; 18:353-65
- Body SC, Shernan SK: Implications of pharmacogenetics for the practice of anesthesiology. *Advances in Anesthesia*. Edited by Lake CL, Johnson JO, McLoughlin TM. Philadelphia, Mosby, 2003, pp 269-96
- Iohom G, FitzGerald D, Cunningham AJ: Principles of pharmacogenetics: Implications for the anaesthetist. *Br J Anaesth* 2004; 93:440-50
- Ziegeler S, Tsusaki BE, Collard CD: Influence of genotype on perioperative risk and outcome. *ANESTHESIOLOGY* 2003; 99:212-9
- Hoehle MR, Timmermann B, Lehrach H: Human inter-individual DNA sequence variation in candidate genes, drug targets, the importance of haplotypes and pharmacogenomics. *Curr Pharm Biotechnol* 2003; 4:351-78
- Jensen FS, Viby-Mogensen J: Plasma cholinesterase and abnormal reaction to succinylcholine: Twenty years' experience with the Danish Cholinesterase Research Unit. *Acta Anaesthesiol Scand* 1995; 39:150-6
- Ostergaard D, Jensen FS, Skovgaard LT, Viby-Mogensen J: Dose-response relationship for mivacurium in patients with phenotypically abnormal plasma cholinesterase activity. *Acta Anaesthesiol Scand* 1995; 39:1016-8
- Cerf C, Mesguish M, Gabriel I, Amselem S, Duvaldestin P: Screening patients with prolonged neuromuscular blockade after succinylcholine and mivacurium. *Anesth Analg* 2002; 94:461-6
- Qin XP, Xie HG, Wang W, He N, Huang SL, Xu ZH, Ou-Yang DS, Wang YJ, Zhou HH: Effect of the gene dosage of CYP2C19 on diazepam metabolism in Chinese subjects. *Clin Pharmacol Ther* 1999; 66:642-6
- Kosuge K, Jun Y, Watanabe H, Kimura M, Nishimoto M, Ishizaki T, Ohashi K: Effects of CYP3A4 inhibition by diltiazem on pharmacokinetics and dynamics of diazepam in relation to CYP2C19 genotype status. *Drug Metab Dispos* 2001; 29:1284-9
- Bertilsson L: Geographical/interracial differences in polymorphic drug oxidation: Current state of knowledge of cytochromes P450 (CYP) 2D6 and 2C19. *Clin Pharmacokinet* 1995; 29:192-209
- Shih PS, Huang JD: Pharmacokinetics of midazolam and 1'-hydroxymidazolam in Chinese with different CYP3A5 genotypes. *Drug Metab Dispos* 2002; 30:1491-6
- Goh BC, Lee SC, Wang LZ, Fan L, Guo JY, Lamba J, Schuetz E, Lim R, Lim HL, Ong AB, Lee HS: Explaining interindividual variability of docetaxel pharmacokinetics and pharmacodynamics in Asians through phenotyping and genotyping strategies. *J Clin Oncol* 2002; 20:3683-90
- Wandel C, Witte JS, Hall JM, Stein CM, Wood AJ, Wilkinson GR: CYP3A activity in African American and European American men: Population differences and functional effect of the CYP3A4*1B5'-promoter region polymorphism. *Clin Pharmacol Ther* 2000; 68:82-91
- Girard T, Urwyler A, Censier K, Mueller CR, Zorzato F, Treves S: Genotype-phenotype comparison of the Swiss malignant hyperthermia population. *Hum Mutat* 2001; 18:357-8
- Stewart SL, Hogan K, Rosenberg H, Fletcher JE: Identification of the Arg1086His mutation in the alpha subunit of the voltage-dependent calcium channel (CACNA1S) in a North American family with malignant hyperthermia. *Clin Genet* 2001; 59:178-84
- Fagerlund TH, Braaten O: No pain relief from codeine? An introduction to pharmacogenomics. *Acta Anaesthesiol Scand* 2001; 45:140-9
- Robinson RL, Brooks C, Brown SL, Ellis FR, Halsall PJ, Quinnell RJ, Shaw MA, Hopkins PM: RYR1 mutations causing central core disease are associated with more severe malignant hyperthermia in vitro contracture test phenotypes. *Hum Mutat* 2002; 20:88-97
- Guis S, Figarella-Branger D, Monnier N, Bendahan D, Kozak-Ribbens G, Mattei JP, Lunardi J, Cozzzone PJ, Pellissier JF: Multiminicore disease in a family susceptible to malignant hyperthermia: histology, in vitro contracture tests, and genetic characterization. *Arch Neurol* 2004; 61:106-13
- Sambuughin N, Sei Y, Gallagher KL, Wyre HW, Madsen D, Nelson TE, Fletcher JE, Rosenberg H, Muldoon SM: North American malignant hyperthermia population: Screening of the ryanodine receptor gene and identification of novel mutations. *ANESTHESIOLOGY* 2001; 95:594-9
- Barone V, Massa O, Intravaia E, Bracco A, Di Martino A, Tegazzin V, Cozzolino S, Sorrentino V: Mutation screening of the RYR1 gene and identification of two novel mutations in Italian malignant hyperthermia families. *J Med Genet* 1999; 36:115-8
- Rueffert H, Olthoff D, Deutrich C, Meinecke CD, Froster UG: Mutation screening in the ryanodine receptor 1 gene (RYR1) in patients susceptible to malignant hyperthermia who show definite IVCT results: identification of three novel mutations. *Acta Anaesthesiol Scand* 2002; 46:692-8
- Rueffert H, Olthoff D, Deutrich C, Thamm B, Froster UG: Homozygous and heterozygous Arg614Cys mutations (1840C->T) in the ryanodine receptor gene co-segregate with malignant hyperthermia susceptibility in a German family. *Br J Anaesth* 2001; 87:240-5
- Manning BM, Quane KA, Ordning H, Urwyler A, Tegazzin V, Lehane M, O'Halloran J, Hartung E, Giblin LM, Lynch PJ, Vaughan P, Censier K, Bendixen D, Comi G, Heytens L, Monsieurs K, Fagerlund T, Wolz W, Heffron JJ, Muller CR, McCarthy TV: Identification of novel mutations in the ryanodine-receptor gene

- (RYR1) in malignant hyperthermia: genotype-phenotype correlation. *Am J Hum Genet* 1998; 62:599-609
27. Kharasch ED, Hankins D, Mautz D, Thummel KE: Identification of the enzyme responsible for oxidative halothane metabolism: Implications for prevention of halothane hepatitis. *Lancet* 1996; 347:1367-71
28. Eliasson E, Gardner I, Hume-Smith H, de W, I, Beaune P, Kenna JG: Interindividual variability in P450-dependent generation of neoantigens in halothane hepatitis. *Chem Biol Interact* 1998; 116:123-41
29. Larrey D, Pageaux GP: Genetic predisposition to drug-induced hepatotoxicity. *J Hepatol* 1997; 26(suppl 2):12-21
30. Ono S, Hatanaka T, Hotta H, Tsutsui M, Satoh T, Gonzalez FJ: Chlorzoxazone is metabolized by human CYP1A2 as well as by human CYP2E1. *Pharmacogenetics* 1995; 5:143-50
31. McCarver DG, Byun R, Hines RN, Hichme M, Wegenek W: A genetic polymorphism in the regulatory sequences of human CYP2E1: Association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. *Toxicol Appl Pharmacol* 1998; 152:276-81
32. Marchand LL, Wilkinson GR, Wilkens LR: Genetic and dietary predictors of CYP2E1 activity: A phenotyping study in Hawaii Japanese using chlorzoxazone. *Cancer Epidemiol Biomarkers Prev* 1999; 8:495-500
33. Campagna JA, Miller KW, Forman SA: Mechanisms of actions of inhaled anesthetics. *N Engl J Med* 2003; 348:2110-24
34. Mueller JL, Ellenberger EA, Vaughn LK, Belknap JK, Quock RM: Detection and mapping of quantitative trait loci that determine responsiveness of mice to nitrous oxide antinociception. *Neuroscience* 2004; 123:743-9
35. Xing Y, Sonner JM, Eger EI, Cascio M, Sessler DI: Mice with a melanocortin 1 receptor mutation have a slightly greater minimum alveolar concentration than control mice. *ANESTHESIOLOGY* 2004; 101:544-6
36. Sonner JM, Gong D, Eger EI: Naturally occurring variability in anesthetic potency among inbred mouse strains. *Anesth Analg* 2000; 91:720-6
37. Selzer RR, Rosenblatt DS, Laxova R, Hogan K: Adverse effect of nitrous oxide in a child with 5,10-methylenetetrahydrofolate reductase deficiency. *N Engl J Med* 2003; 349:45-50
38. Erbe RW, Salis RJ: Severe methylenetetrahydrofolate reductase deficiency, methionine synthase, and nitrous oxide: A cautionary tale. *N Engl J Med* 2003; 349:5-6
39. Bond C, LaForge KS, Tian H, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L: Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: Possible implications for opiate addiction. *Proc Natl Acad Sci U S A* 1998; 95:9608-13
40. Lotsch J, Zimmermann M, Darimont J, Marx C, Dudziak R, Skarke C, Geisslinger G: Does the A118G polymorphism at the μ -opioid receptor gene protect against morphine-6-glucuronide toxicity? *ANESTHESIOLOGY* 2002; 97:814-9
41. Skarke C, Darimont J, Schmidt H, Geisslinger G, Lotsch J: Analgesic effects of morphine and morphine-6-glucuronide in a transcutaneous electrical pain model in healthy volunteers. *Clin Pharmacol Ther* 2003; 73:107-21
42. Uhl GR, Sora I, Wang Z: The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. *Proc Natl Acad Sci U S A* 1999; 96:7752-5
43. Sawyer MB, Innocenti F, Das S, Cheng C, Ramirez J, Pantle-Fisher FH, Wright C, Badner J, Pei D, Boyett JM, Cook E Jr, Ratain MJ: A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. *Clin Pharmacol Ther* 2003; 73:566-74
44. Poulsen L, Brosen K, Arendt-Nielsen L, Gram LF, Elbaek K, Sindrup SH: Codeine and morphine in extensive and poor metabolizers of sparteine: Pharmacokinetics, analgesic effect and side effects. *Eur J Clin Pharmacol* 1996; 51:289-95
45. Ingelman-Sundberg M: Duplication, multiduplication, and amplification of genes encoding drug-metabolizing enzymes: Evolutionary, toxicological, and clinical pharmacological aspects. *Drug Metab Rev* 1999; 31:449-59
46. Eckhardt K, Li S, Ammon S, Schanzle G, Mikus G, Eichelbaum M: Same incidence of adverse drug events after codeine administration irrespective of the genetically determined differences in morphine formation. *Pain* 1998; 76:27-33
47. Eichelbaum M, Kroemer HK, Fromm MF: Impact of P450 genetic polymorphism on the first-pass extraction of cardiovascular and neuroactive drugs. *Adv Drug Deliv Rev* 1997; 27:171-99
48. Bachs L, Skurtveit S, Morland J: Codeine and clinical impairment in samples in which morphine is not detected. *Eur J Clin Pharmacol* 2003; 58:785-9
49. Levo A, Koski A, Ojanpera I, Vuori E, Sajantila A: Post-mortem SNP analysis of CYP2D6 gene reveals correlation between genotype and opioid drug (tramadol) metabolite ratios in blood. *Forensic Sci Int* 2003; 135:9-15
50. Stamer UM, Lehnen K, Hothker F, Bayerer B, Wolf S, Hoefl A, Stuber F: Impact of CYP2D6 genotype on postoperative tramadol analgesia. *Pain* 2003; 105:231-8
51. Eap CB, Broly F, Mino A, Hammig R, Deglon JJ, Uehlinger C, Meili D, Chevalley AF, Bertschy G, Zullino D, Kosel M, Preisig M, Baumann P: Cytochrome P450 2D6 genotype and methadone steady-state concentrations. *J Clin Psychopharmacol* 2001; 21:229-34
52. Shiran MR, Chowdry J, Rostami-Hodjegan A, Ellis SW, Lennard MS, Iqbal MZ, Lagundoye O, Seivewright N, Tucker GT: A discordance between cytochrome P450 2D6 genotype and phenotype in patients undergoing methadone maintenance treatment. *Br J Clin Pharmacol* 2003; 56:220-4
53. Iribarne C, Berthou F, Baird S, Dreano Y, Picart D, Bail JP, Beaune P, Menez JF: Involvement of cytochrome P450 3A4 enzyme in the N-demethylation of methadone in human liver microsomes. *Chem Res Toxicol* 1996; 9:365-73
54. Foster DJ, Somogyi AA, Bochner F: Methadone N-demethylation in human liver microsomes: Lack of stereoselectivity and involvement of CYP3A4. *Br J Clin Pharmacol* 1999; 47:403-12
55. Guillon J, Buronfosse T, Desage M, Lepape A, Brazier JL, Beaune P: Possible involvement of multiple cytochrome P450s in fentanyl and sufentanil metabolism as opposed to alfentanil. *Biochem Pharmacol* 1997; 53:1613-9
56. Kharasch ED, Russell M, Mautz D, Thummel KE, Kunze KL, Bowdle A, Cox K: The role of cytochrome P450 3A4 in alfentanil clearance: Implications for interindividual variability in disposition and perioperative drug interactions. *ANESTHESIOLOGY* 1997; 87:36-50
57. Kest B, Sarton E, Dahan A: Gender differences in opioid-mediated analgesia: Animal and human studies. *ANESTHESIOLOGY* 2000; 93:539-47
58. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppel RA, Stohler CS, Goldman D: COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science* 2003; 299:1240-3
59. Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melen K, Julkunen I, Taskinen J: Kinetics of human soluble and membrane-bound catechol O-methyltransferase: A revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 1995; 34:4202-10
60. Vandenberg DJ, Rodriguez LA, Miller IT, Uhl GR, Lachman HM: High-activity catechol-O-methyltransferase allele is more prevalent in polysubstance abusers. *Am J Med Genet* 1997; 74:439-42
61. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR: Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 2001; 98:6917-22
62. LaForge KS, Yuforov V, Kreek MJ: Opioid receptor and peptide gene polymorphisms: potential implications for addictions. *Eur J Pharmacol* 2000; 410:249-68
63. Kreek MJ, Nielsen DA, LaForge KS: Genes associated with addiction: Alcoholism, opiate, and cocaine addiction. *Neuromolecular Med* 2004; 5:85-108
64. Sandberg M, Yasar U, Stromberg P, Hoog JO, Eliasson E: Oxidation of celecoxib by polymorphic cytochrome P450 2C9 and alcohol dehydrogenase. *Br J Clin Pharmacol* 2002; 54:423-9
65. Kirchheiner J, Stormer E, Meisel C, Steinbach N, Roots I, Brockmoller J: Influence of CYP2C9 genetic polymorphisms on pharmacokinetics of celecoxib and its metabolites. *Pharmacogenetics* 2003; 13:473-80
66. Tracy TS, Hutzler JM, Haining RL, Rettie AE, Hummel MA, Dickmann LJ: Polymorphic variants (CYP2C9*3 and CYP2C9*5) and the F114L active site mutation of CYP2C9: Effect on atypical kinetic metabolism profiles. *Drug Metab Dispos* 2002; 30:385-90
67. Tang C, Shou M, Rushmore TH, Mei Q, Sandhu P, Woolf EJ, Rose MJ, Gelmann A, Greenberg HE, De L, I, Van Hecken A, De Schepper PJ, Ebel DL, Schwartz JJ, Rodrigues AD: In-vitro metabolism of celecoxib, a cyclooxygenase-2 inhibitor, by allelic variant forms of human liver microsomal cytochrome P450 2C9: Correlation with CYP2C9 genotype and in-vivo pharmacokinetics. *Pharmacogenetics* 2001; 11:223-35
68. Kirchheiner J, Meineke I, Freytag G, Meisel C, Roots I, Brockmoller J: Enantiospecific effects of cytochrome P450 2C9 amino acid variants on ibuprofen pharmacokinetics and on the inhibition of cyclooxygenases 1 and 2. *Clin Pharmacol Ther* 2002; 72:62-75
69. Lee CR, Pieper JA, Frye RF, Hinderliter AL, Blaisdell JA, Goldstein JA: Tolbutamide, flurbiprofen, and losartan as probes of CYP2C9 activity in humans. *J Clin Pharmacol* 2003; 43:84-91
70. Yasar U, Eliasson E, Forslund-Bergengren C, Tybring G, Gadd M, Sjoqvist F, Dahl ML: The role of CYP2C9 genotype in the metabolism of diclofenac in vivo and in vitro. *Eur J Clin Pharmacol* 2001; 57:729-35
71. Brenner SS, Herrlinger C, Dilger K, Murdert TE, Hofmann U, Marx C, Klotz U: Influence of age and cytochrome P450 2C9 genotype on the steady-state disposition of diclofenac and celecoxib. *Clin Pharmacokinet* 2003; 42:283-92
72. Aithal GP, Day CP, Leathart JB, Daly AK: Relationship of polymorphism in CYP2C9 to genetic susceptibility to diclofenac-induced hepatitis. *Pharmacogenetics* 2000; 10:511-8
73. Ueshima Y, Tsutsumi M, Takase S, Matsuda Y, Kawahara H: Acetaminophen metabolism in patients with different cytochrome P-4502E1 genotypes. *Alcohol Clin Exp Res* 1996; 20:25A-8A
74. Bernal W, Donaldson P, Underhill J, Wendon J, Williams R: Tumor necrosis factor genomic polymorphism and outcome of acetaminophen (paracetamol)-induced acute liver failure. *J Hepatol* 1998; 29:53-9
75. Quirarte J, Sanchez-Garcia F, Torres MJ, Blanco C, Castillo R, Ortega N, de Castro FR, Perez-Aciego P, Carrillo T: Association of HLA-DR11 with the anaphylactoid reaction caused by nonsteroidal anti-inflammatory drugs. *J Allergy Clin Immunol* 1999; 103:685-9
76. Fritsche E, Baek SJ, King LM, Zeldin DC, Eling TE, Bell DA: Functional characterization of cyclooxygenase-2 polymorphisms. *J Pharmacol Exp Ther* 2001; 299:468-76
77. Cipollone F, Toniato E, Martinotti S, Fazio M, Iezzi A, Cucurullo C, Pini B, Ursi S, Vitullo G, Averna M, Arca M, Montali A, Campagna F, Uccchino S, Spigonardo F, Taddei S, Virdis A, Ciabattoni G, Notarbartolo A, Cucurullo F, Mezzetti A: A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. *JAMA* 2004; 291:2221-8