

Pharmacogenomic Variability and Anaesthesia

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INTRODUCTION

PHARMACOGENETICS AND PHARMACOGENOMICS IN ANAESTHESIA

“If it were not for the great variability among individuals, medicine might as well be a science and not an art” – Sir William Osler 1892

The practice of anaesthesia has long been considered an art and a science; with inter patient variability in drug response being the rule, rather than the exception. Differences in patient response have historically been attributed to factors such as age, sex, pre-existing disease, drug interactions, surgery type and nutritional status.

Anaesthesia has been at the forefront in the discovery of pharmacogenetic disorders such as pseudocholinesterase deficiency, malignant hyperthermia and thiopental induced porphyria. These problems were described in the 1960s whose investigation helped craft the developing new science of pharmacogenomics. Perhaps today we take it for granted that the aforementioned conditions are genetically based problems.

Since the unravelling of the structure of DNA by Watson and Crick half a century ago, the scientific community has plunged into a head long quest to unlock the secrets of the human genome which culminated in the recent successful completion of the Human Genome Project. Among the surprises which accompanied the completion of this enormous task, were the relative paucity of genes identified in comparison to other species a mere 32000 compared with 19000 of the tiny nematode.

Secondly the extent of redundant or non-coding sequence sometimes referred to as junk DNA and thirdly the relative variation between individuals or among racial groups. More surprising perhaps is the similarity between Homo sapiens and other species. For example the genome of man and the chimpanzee are 98.8% identical, 75% of the dogs genome is shared with man and even lower life forms have sizable length of DNA which are identical to areas in the human genome.

The terms pharmacogenetic and pharmacogenomic are often used interchangeably, pharmacogenetics generally refers to the variation of a single gene or relatively few genes influencing the expression of drug response, whereas pharmacogenomics refers to genome wide alterations in drug response.

The meddling of pharmacology and genetics termed pharmacogenetics coined in 1959 by Frederick Vogel occurred in the 1950s primarily due to earlier clinical observation, land mark reports based on these observations and the development of new laboratory and experimental techniques. Werner Kalow published the first monograph in 1962, entitled Pharmacogenetics: Heredity and

Response to drugs, a land mark document that contributed to the elevation of pharmacogenetics to a respected science and discipline.

SOME IMPORTANT MILESTONES IN THE HISTORY OF PHARMACOGENOMICS

Date Author Discovery

1866 Mendel Lays down the principles of heredity

1909 Garrod Publication of 'Inborn Errors of Metabolism'

1932 Snyder Characterization of the phenylthiourea-non-taster as an autosomal recessive trait

1954 Hughes et al. Relates isoniazid neuropathy to metabolism

1956 Carson et al. Discovery of glucose G-6 PD deficiency

1957 Kalow Characterizes acetylcholinesterase deficiency

1957 Motulsky Explanation for the inherited differences in drug metabolism

1957 Vogel Coins the term 'pharmakogenetik'

1960 Price Evans Characterization of acetylators polymorphisms

1962 Kalow The first textbook on pharmacogenetics

1977 Mahgoub et al. Description of the debrisoquine polymorphism

1979 Eichelbaum et al. Describes sparteine metabolism polymorphism

1980 Weinshilboum & Sladek Description of genetics of mercaptopurine metabolism

1982 Eichelbaum et al. Recognition of link between sparteine and debrisoquine metabolism

1984 Wedlund et al. Description of the cytochrome CYP2C19 polymorphism

1988 Gonzalez Explanation for the debrisoquine phenotype

1992 Grant & Meyer Explanation of the molecular genetics of NAT

1997 Yates et al. Polymerase chain reaction (PCR) based methods used to detect thiopurine S-methyl transferase (TPMT) deficiency

Pharmacogenomics has the potential to individualize drug therapy, help avoid adverse drug reactions and toxic effects, and improve therapeutic drug efficacy and outcomes by adjusting drug therapy to the patient's genotype. Although a perfectly tailored therapy in every case may not currently exist the concept of personalized medicine is to bring this concept to reality.

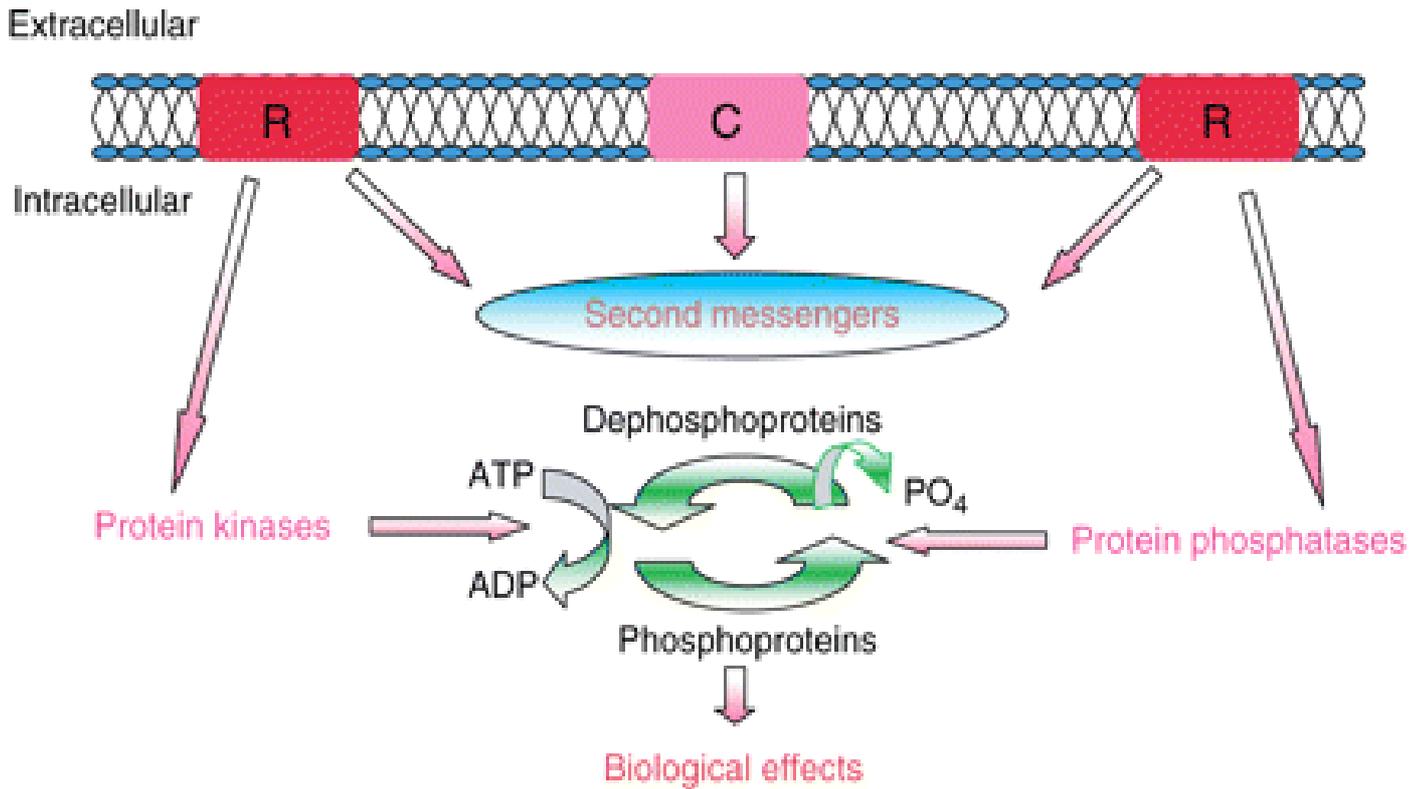
According to the US Food and Drug Administration domestically there are more than 2 million serious adverse drug reactions yearly of which there are 10 000 deaths. For patients experiencing adverse drug reactions costs increase in terms of average length of hospital stay and mortality. The application of pharmacogenomics has the potential to help providers reduce the number of adverse drug reactions patients experience, improve therapeutic efficacy and prevent adverse drug reactions.

The effect of drugs on gene expression can be studied with genomic technologies, which provide an additional perspective of the biological effects of a drug. Currently the pharmacogenomic impact of polymorphisms, which are genetic alterations and occur in more than 1% of the population, is most noted among 3 categories: enzymes, transporter proteins and receptors.

Genetically determined pharmacodynamic variability can be mediated at several stages of drug action:

1. Enzymes: inter-individual variation in therapeutic drug response and toxicity is most often a result of variability in drug metabolism rather than pharmacodynamics. Such genes generally affect drug transformation by altering the amount or function of an enzyme.
2. Transporter proteins: genetic variability influences drug absorption and this forms the basis for slow and rapid drug absorption. Some drugs are actively transported by transporter proteins of which membrane transporters play a role.
3. Receptors: when examining the response to a drug, the most obvious target for genetic studies of drug response is the receptor.

Fig 1.



C- Ion channels

R - Receptors

Malignant hyperthermia (MH)

The rare hypermetabolic syndrome, MH, is an inherited genetic variation. It is a life threatening condition that occurs when a susceptible person is exposed to triggers e.g. halogenated inhalation agents and/or succinylcholine. The triggers cause a rapid release of calcium from the sarcoplasmic reticulum, resulting in a high metabolic state, increased oxygen consumption, hypercapnia and increased body temperature. Left untreated MH can lead to circulatory collapse and death. The incidence varies from 1: 5000 to 1:50 000 in adults and around 1:15 000 in children.

Two disorders are associated with MH: Central core disease and King-Denborough Syndrome. Specific signs of MH include increase end-tidal carbon dioxide (early sign), marked temperature elevation (late sign), muscle rigidity (may or may not be present, myoglobinuria and rhabdomyolysis (muscle breakdown). Non-specific signs of MH include tachycardia, acidosis, hyperkalaemia and tachypnoea.

There are almost 50 mutations that have been found associated with MH although the RYR1 gene mutation on chromosome 19 is the most predominant. Ryanodine

receptors mediate calcium release from the sarcoplasmic reticulum, which is an essential step in muscle contraction. In MH the release of calcium from the sarcoplasmic reticulum outweighs the uptake resulting in an inability to terminate muscle contraction.

Once MH is recognized it is imperative to act quickly to discontinue the inhalational agent, hyperventilate with 100% oxygen and administer dantrolene, cool patient and treat symptoms. Dantrolene decreases the release of calcium from the sarcoplasmic reticulum and restoring the balance between the release and re-uptake.

The contracture halothane caffeine test is the most common diagnostic test for the diagnosis of MH. This test requires muscle biopsy and measures contracture in response to caffeine and halothane. The test is very sensitive meaning most people with the risk and susceptibility for MH will be identified. Problems with the halothane contracture test include the requirement for a fresh sample at the testing site, the invasiveness and expense of the test and limited biopsy centres. Mortality from MH has decreased from 80% thirty years ago to less than 5% today.

Abnormalities in butyrylcholinesterase

The level and quality of butyrylcholinesterase BChE also known as plasma cholinesterase in a patient determines the duration of action of succinylcholine and mivacurium. Inherited deficiency or reduced efficacy of plasma cholinesterase will result in prolonged muscle relaxation after succinylcholine. This was the first documented example of inherited variation in anaesthetic drug effects.

The gene encoding for plasma cholinesterase has been localized to a single autosomal location on the long arm of chromosome 3, more specifically 3q 26-1 – q26-2. Genetic variation is only one of several factors determining the activity of cholinesterase in plasma. A few years ago there were only few known forms of human serum BChE, coded for by the Usual U, Atypical A, Fluoride-resistant F and Silent S alleles.

Today over 50 genetic variants identifiable at DNA level, the complexity of diagnosis and the interpretation of these genetic traits has greatly increased.

- U : the usual variant 96% of the population are U homozygotes
- A : atypical or dibucaine-resistant variant
- K : the Kalow variant has two thirds of the activity of the U variant
- S: the silent variant has no butyrylcholinesterase activity.

Variants may exhibit quantitative alterations in enzymatic activity such as H, J and K alleles or qualitative variants such as the A, F (Fluoride resistant) and S (silent) alleles.

Although the presence of a single genetic variant allele does not usually cause an increased duration of action of succinylcholine or mivacurium, it may do so if it occurs in heterozygous combination with otherwise induced low BChE activity such as with concurrent illness or anticholinesterase administration.

Cholinesterase deficiency is usually due to homozygosity for the abnormal allele, the enzyme produced by the homozygous state is usually altered both quantitatively and qualitatively.

Quantitative variants: the K variant is caused by a point mutation, resulting in the substitution of Alanine to Threonine at position 539 in the amino acid sequence of the subunit. The K variant is the most common clinically significant BChE variant. It has recently been shown that patients with a K variant have a significantly prolonged duration of action of mivacurium 30-40% if U/K genotype.

The J variant is associated with a 33% reduced concentration of BChE.

Qualitative variant

The A variant is a result of a point mutation resulting in Aspartamine being substituted by Glycine at position 70. Patients who are homozygous for the A variant are very sensitive to mivacurium and suxamethonium and extremely long duration of action has been reported after 0.12 – 0.2 mg/kg - the time for full spontaneous recovery of neuromuscular function is 6-8 hr, compared to 30 minutes in patients with normal BChE. In the case of suxamethonium the effect may persist for over 2hrs. The F allele has reduced activity in vivo and demonstrates fluoride inhibition.

The S variant results from a stop codon. It occurs because of multiple mutations and has no activity. Neuromuscular blockade may last for several hours in the homozygote.

Confirmatory tests

Current testing of patients and their families who are suspected to have scoline apnoea relies on ascertaining the dibucaine number. If plasma from the patient with normal BChE is added to a water bath containing benzoyl choline, a chemical reaction occurs.

The reaction emits light which can be measured spectrophotometrically and if dibucaine is added the reaction is inhibited and no light is produced. Dibucaine is a local anaesthetic that inhibits normal BChE activity by 80% but only inhibits the

homozygous atypical enzyme by 20%. Heterozygous atypical enzyme by 20%. Heterozygotes are characterized by an intermediate percentage of inhibition 40 – 60%. The percentage of inhibition of BChE by dibucaine is termed the dibucaine number

Prophyria

It's a group of genetic disorders characterized by partial deficiencies in the haem biosynthesis pathway. The deficiency results in the accumulation of haem precursors and porphyrins. Symptomatology is dependent on the specific enzyme that is deficient and on the type of porphyria that subsequently accumulate.

World wide the commonest acute porphyria is Acute Intermittent Porphyria (AIP) with an incidence of 1:20 000 in Europe. In South Africa however, Varigate Porphyria is the commonest with an incidence of around 1: 250 to 1:500 among the Afrikaner population.

The control of haem production is tightly regulated through feedback inhibition of ALA synthesis so the levels of haem closely match demand. Attacks are precipitated by events that reduce haem concentration.

This leads to an increase in ALA synthetase activity which results in an increased level of porphyria precursors. Acute attacks are characterized by autonomic instability, neuropsychiatric dysfunction, severe abdominal pain and electrolyte disturbances and may prove fatal in the extreme.

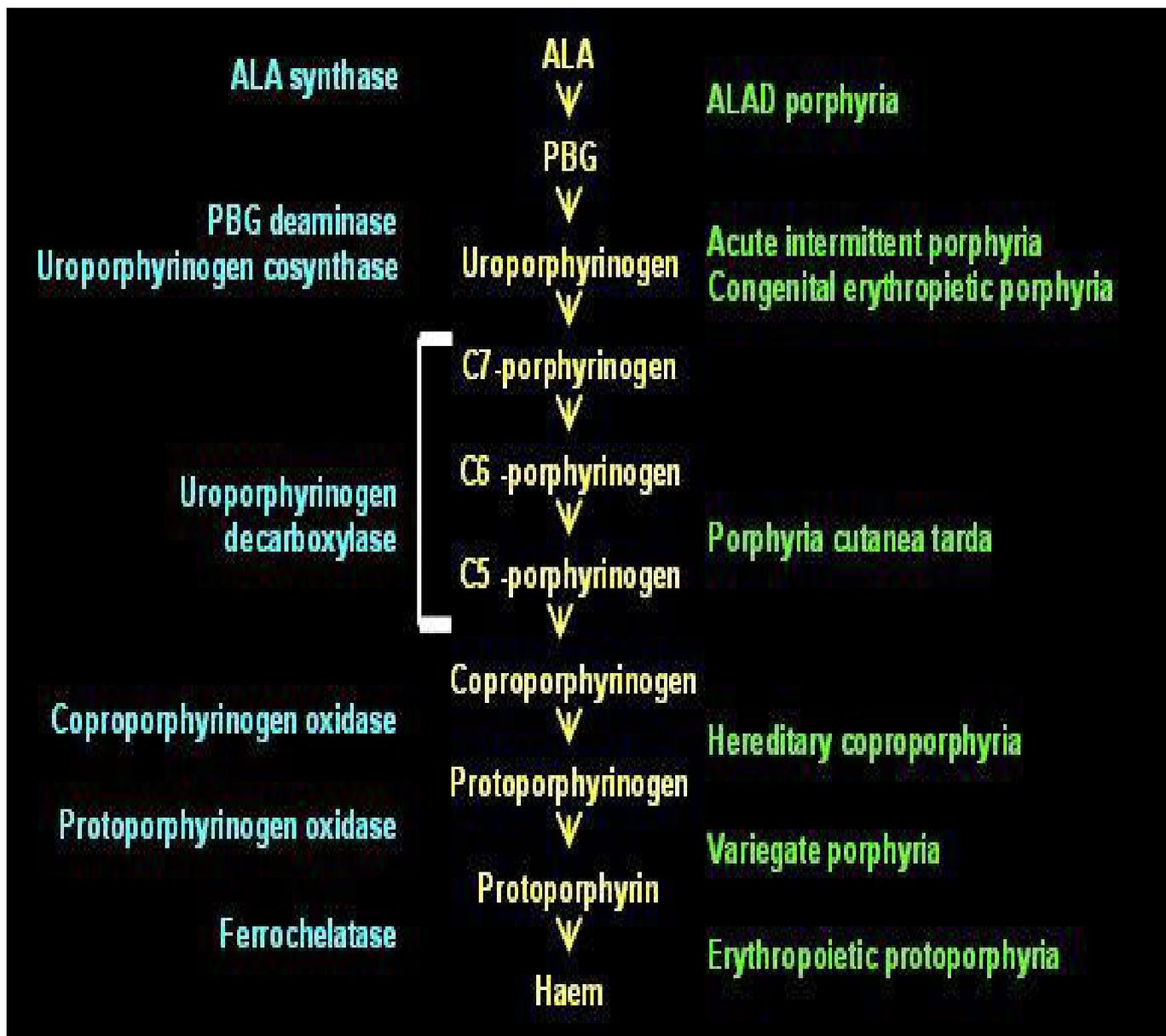
The implications for the anaesthetist is that the disease may be dormant until periods of stress including surgery and illness or by the administration of drugs in particular barbiturates (thiopentone).

AIP is caused by a deficiency in porphobilinogen PBG deaminase. It is an autosomal dominant disease with the gene loci on chromosome 11. It is of primary importance to the anaesthetist as it is the porphyria in which an acute attack is most likely to prove fatal.

Variagate porphyria is due to a deficiency in protoporphyrinogen oxidase. The gene for this enzyme is found on chromosome 1. The genes encoding the deficient enzymes in hereditary and plumboporphyria are both situated on chromosome 9.

DIAGRAM OF HEAM SYNTHESIS PATHWAY

Fig 2.



Treating an acute crises

In the event of an acute crises, it is vital to assess muscle strength and bulbar function as severe disease may cause respiratory failure and a risk of aspiration. Post operative ventilation may be necessary. All possible precipitants must be withdrawn and adequate hydration must be addressed.

Haematin is the only specific treatment that probably has an effect by supplementing the free haem pool and suppressing ALA synthetase. However it may cause renal failure, coagulopathy and thrombophlebitis. Haem arginate is a suitable alternative that is devoid of the side effects associated with haematin.

Beta blockers have a dual role, both controlling tachycardia and decreasing the activity of ALA synthase. In the ICU setting it is recommended that a high carbohydrate intake should be instituted as carbohydrate loading can suppress porphyrin synthesis

Drugs considered safe

- Nitrous oxide, halothane
- Propofol
- Aspirin, codeine, ibuprofen
- Pancuronium, suxamethonium
- Neostigmine
- Bupivacaine
- Fentanyl, pethidine, morphine

Use with caution

- Isoflurane, enflurane, sevoflurane
- Ketamine
- Atracurium
- Midazolam
- Tramadol

Use with extreme caution

- Diclofenac, ketorolac, tilidine
- Quinolones
- Amlodipine
- Nitrazepam

Avoid

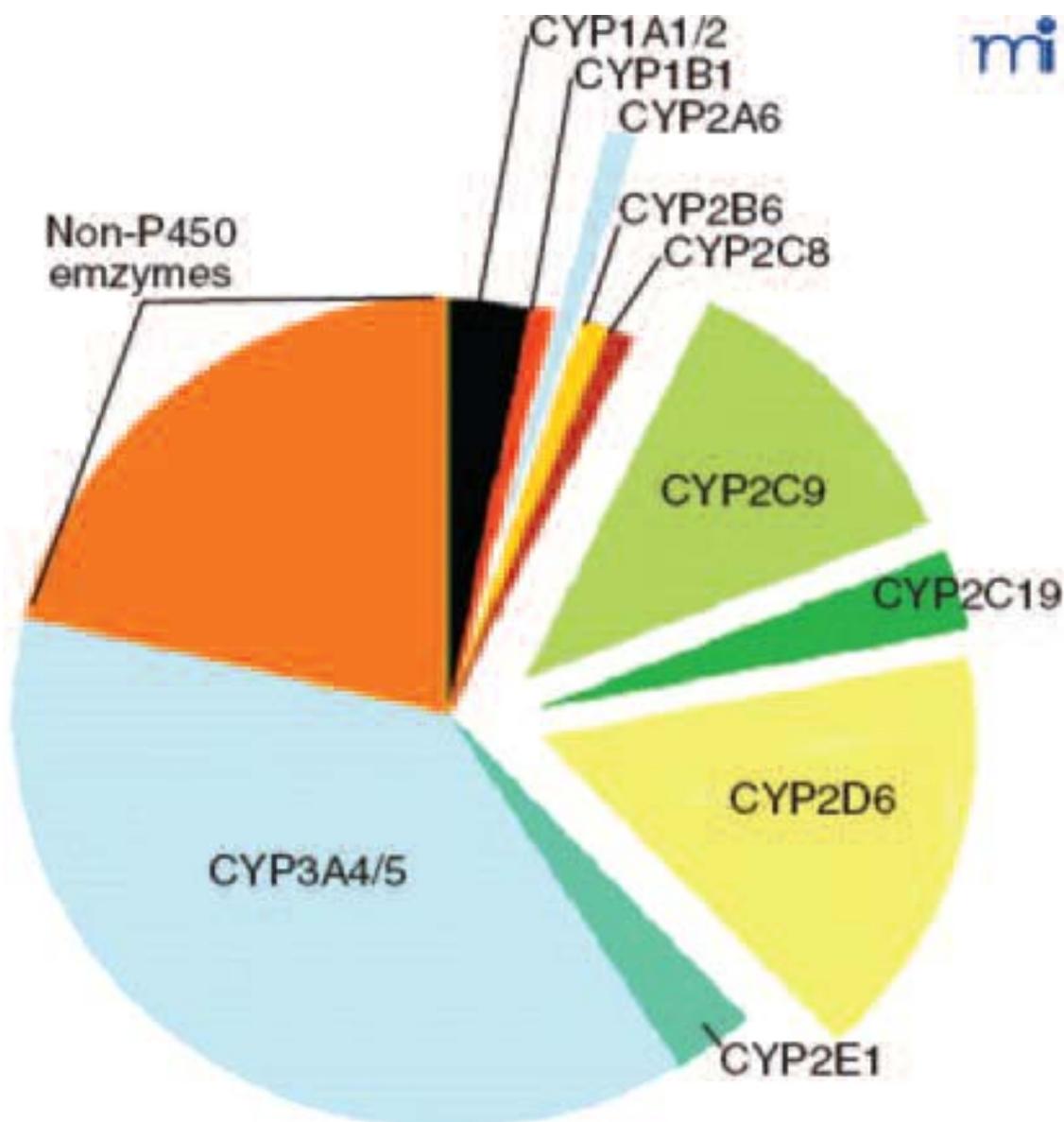
- Thiopentone
- Ropivacaine
- Pentazocine
- Clindamycin, erythromycin

THE CYTOCHROME P450

Drug metabolism involves a transformation process in the liver, these reaction are termed phase 1 and phase 2. Phase 1 reactions occur by oxidation, reduction, or hydrolysis and make the metabolite more polar or water soluble to facilitate renal excretion. Phase 2 involves conjugation which also makes the drug metabolite more polar and thus helps facilitate excretion in the urine.

Cytochrome P450 is used to describe a family of microsomal drug metabolizing enzymes that are responsible for 70% to 80% of Phase 1 metabolism of medication.

Fig 3 . Contribution of Major Human P450 Phase 1 Metabolism of All Drugs Currently Markete



Cytochrome P450 enzymes are important in the biosynthesis and metabolism of endogenous compounds such as vitamins, steroids and lipids and the majority of these processes take place in the liver and less frequently in the epithelium of the small intestines.

There are 57 different CYP450 genes identified in humans, but only a relatively small number of the encoded proteins primarily CYP1, CYP2 and CYP3 families appear to contribute to conjugation of drug. The clinical relevance of the inherited variations associated with CYP450 enzymes is dependent on many environmental factors such as disease, drugs, surgery, nutritional status and biological variations. The frequency of variant alleles is also significantly varied depending on race and ethnic background.

A unique feature of the hepatic microsomal enzymes is the capability of medications or chemicals to stimulate or induce activity levels of these enzymes. Examples of agents that induce activity levels of hepatic microsomal enzymes are Phenobarbital, St. John's wort, polycyclic hydrocarbons and rifamycins. Stimulation of these enzymes increases the rate of drug metabolism on the other hand; certain medication and substances such as calcium channel blockers, grape fruit, and erythromycin inhibit microsomal enzymes. These inhibitors and inducers add to the variability of the CYP450 and possible adverse drug effects.

The major CYP450 enzymes of importance to anaesthesia providers are: CYP3A4, CYP2D6, CYP2C9 and CYP 2C19.

The CYP2E1 is responsible for the metabolism of inhalational agents, although variability has not been associated with genetic polymorphism but rather with body mass, diet, alcohol consumption and age.

The CYP3A4 family is responsible for the oxidative metabolism of over half of all medications that undergo phase 1 metabolism. This enzyme is found in large quantities in the epithelial layer of the small intestines and also in the liver. CYP3A4 has the ability to metabolize a variety of drugs from many different classes: opioids, benzodiazepines, local anaesthetics, immunosuppressants and antihistamines.

There is appreciable amount of genetic variability within this enzyme, yet interestingly its distribution is continuous and unimodal and has few known clinically significant variations. Although dose adjustments are generally not needed for CYP3A4 polymorphisms, drugs metabolized through CYP3A4 are subject to inhibitors such as cimetidine, ketoconazole and grapefruit juice. This inhibition leads to reduced CYP3A4.

Another important member of the CYP450 family is CYP2D6, which is noted for extensive variability in the enzymes and its effects in the metabolism of many drugs currently in use in anaesthesia practice.

CYP2D6 is responsible for around 25% of phase 1 drug reactions. There may be as much as a 1000 fold difference in the metabolism of drugs by CYP2D6 between phenotypes which may result in adverse drug reactions in patients with a polymorphism of the CYP2D6 enzyme. The CYP2D6 enzyme is responsible for metabolism of many anti emetics, beta blockers, codeine, tramadol, oxycodone, and hydroxycodone, tamoxifen, anti depressants, neuroleptics and anti arrhythmic.

Testing is available to categorize a person's CYP2D6 metabolism as poor, intermediate, extensive (normal) and ultra rapid, and these designations can be helpful for clinicians to evaluate the efficacy and dosages of many medications commonly used in breast cancer treatment and psychiatry. Although testing may be beneficial in anaesthesia, associated adjustments in dosing and clear recommendations have not been fully developed and have not become standard practice.

People with poor metabolism have non-functional or non-existent CYP2D6 enzymes and people with ultra rapid metabolism have overactive enzymes. Therefore people who take medications metabolized by CYP2D6 and who have poor CYP2D6 metabolism may have decreased effect of a drug due to a low plasma concentration.

Drugs metabolized by CYP2D9 include phenytoin, NSAIDs, celecoxib and warfarin. Testing is available for CYP2D9 and several studies have shown that performing and evaluating CYP2D9 genotyping can be helpful in providing safe, more personalized warfarin treatment and to reach therapeutic levels sooner while reducing potentially life-threatening side effects.

CYP2C19 metabolisms include phenytoin, amitriptyline, barbiturates, diazepam and proton pump inhibitors.

IMPLICATIONS OF GENETICS FOR PRE-OPERATIVE ASSESSMENT

Coagulation

Evaluation of pre-operative patients often involves assessment of therapeutic anticoagulation. Balancing the risks of thrombosis against bleeding is a fundamental patient safety issue. No other drug better epitomizes the concern for drug safety than warfarin. I

n a study of adverse drug events leading to emergency room visits in the USA warfarin was the biggest offender 17.3% of all events. Over 30 genes are involved in the mechanism of warfarin-mediated anticoagulation. Notable are CYP2C9 and VKORC1, which affect pharmacokinetics and pharmacodynamics respectively and account for more than 50% of the interindividual variability in dosage.

Small trials have recently been performed and large prospective trials are ongoing in the US and Europe to test whether algorithms for warfarin dosage that use genetic information improve outcome for example better control and a shorter time to achieving a stable dose.

The international Warfarin Pharmacogenetic Consortium comprises 21 research groups from 9 countries and 4 continents. The research group contributed clinical and genetic data for a total of 5700 patients who were treated with warfarin. Using the validation set, the final model they compared dose prediction from the pharmacogenetic group with those from two other models: a clinical model that did not include genetic factors and a model with a fixed dose of 5mg of warfarin per day.

They also assessed performance of the algorithm in three dose groups participating requiring a low dose < 21mg per week, those requiring a high dose >49mg per week and those requiring intermediate doses >21 and <49mg per week for stable therapeutic anticoagulation.

Table 1**Table 2. Predicted Warfarin Doses with the Pharmacogenetic Algorithm, Clinical Algorithm, and Fixed-Dose Approach as Compared with the Actual Stable Dose in the Derivation and Validation Cohorts.***

Prediction Model	Derivation Cohort		Validation Cohort†	
	Mean Absolute Error (95% CI)	R ²	Mean Absolute Error (95% CI)	R ²
	mg/wk	%	mg/wk	%
Pharmacogenetic algorithm‡§	8.3 (8.1–8.6)	47	8.5 (8.0–9.0)	43
Clinical algorithm§	10.0 (9.7–10.3)	27	9.9 (9.3–10.4)	26
Fixed-dose approach¶	13.3 (13.0–13.5)	0	13.0 (12.4–13.6)	0

* The 95% confidence intervals (CIs) on the estimates of mean absolute error were computed by bootstrapping with 1000 replications. R² is the coefficient of determination.

† In the calculation of the mean absolute error in the validation cohort, data from one patient who was taking an unusually high dose of warfarin were excluded.

‡ P<0.001 for the pharmacogenetic algorithm as compared with the clinical algorithm, as derived with the use of McNemar's test of paired proportions.

§ P<0.001 for the pharmacogenetic algorithm as compared with the fixed-dose approach and for the clinical algorithm as compared with the fixed-dose approach, as derived with the use of McNemar's test of paired proportions.

¶ The fixed dose was 35 mg of warfarin per week.

Table 2

Table 3. Percentage of Patients in the Validation Cohort and in the Derivation-plus-Validation Cohort with an Ideal, Underestimated, or Overestimated Dose of Warfarin, as Estimated with the Pharmacogenetic Algorithm, Clinical Algorithm, and Fixed-Dose Approach in Patients Requiring Low, Intermediate, or High Actual Doses of Warfarin for a Therapeutic Effect.*

Actual Dose Required	No. of Patients	Ideal Dose		Underestimation		Overestimation	
		Percent	P Value†	Percent	P Value†	Percent	P Value†
Validation cohort only							
≤21 mg/wk	324						
Pharmacogenetic approach		33.0	0.008, <0.001	4.6	0.002, <0.001	62.3	<0.001, <0.001
Clinical approach		25.9	<0.001	0.6	0.50	73.5	<0.001
Fixed-dose approach		0		0		100	
>21 mg/wk to <49 mg/wk	560						
Pharmacogenetic approach		54.6	0.72, 0.31	26.8	0.14, <0.001	18.6	0.25, <0.001
Clinical approach		53.6	0.55	29.8	<0.001	16.6	<0.001
Fixed-dose approach		51.6		9.1		39.3	
≥49 mg/wk	125						
Pharmacogenetic approach		36.8	<0.001, <0.001	63.2	<0.001, <0.001	0	1.00, 1.00
Clinical approach		9.6	<0.001	89.6	<0.001	0.8	1.00
Fixed-dose approach		0		100		0	
Derivation-plus-validation cohort							
≤21 mg/wk	1711						
Pharmacogenetic approach		35.0	<0.001, <0.001	5.4	<0.001, <0.001	59.7	<0.001, <0.001
Clinical approach		24.0	<0.001	1.2	<0.001	74.8	<0.001
Fixed-dose approach		0		0		100	
>21 mg/wk to <49 mg/wk	2716						
Pharmacogenetic approach		55.9	0.02, 0.08	25.9	<0.001, <0.001	18.2	<0.001, <0.001
Clinical approach		53.3	0.80	31.0	<0.001	15.6	<0.001
Fixed-dose approach		53.7		8.9		37.4	
≥49 mg/wk	625						
Pharmacogenetic approach		32.8	<0.001, <0.001	66.7	<0.001, <0.001	0.5	1.00, 0.25
Clinical approach		13.3	<0.001	86.2	<0.001	0.5	0.25
Fixed-dose approach		0		100		0	

Table 3

Table 4. Ability of the Pharmacogenetic and Clinical Algorithms to Correctly Identify Patients Requiring Low or High Doses of Warfarin.				
Actual or Predicted Dose Group	≤21 mg/wk		≥49 mg/wk	
	Pharmacogenetic Algorithm	Clinical Algorithm	Pharmacogenetic Algorithm	Clinical Algorithm
Validation cohort				
No. of patients predicted to require extreme dose	232	162	48	15
No. of patients actually requiring extreme dose	324	324	125	125
No. of patients correctly predicted to require extreme dose	160*	108	31*	9
Percent of patients correctly predicted to require extreme dose†	49.4	33.3	24.8	7.2
Derivation-plus-validation cohort				
No. of patients predicted to require extreme dose	1250	829	243	98
No. of patients actually requiring extreme dose	1711	1711	625	625
No. of patients correctly predicted to require extreme dose	929*	571	165	57
Percent of patients correctly predicted to require extreme dose†	54.3	33.4	26.4	9.1

The pharmacogenetic algorithm provided dose estimates that were significantly closer to the actual doses required than the estimates derived from the clinical algorithm or the fixed dose approach as evidenced by an absolute error that was lower than that for both the clinical algorithm and the fixed dose approach. Overall estimates of dose derived with the use of the pharmacogenetic algorithm were closer to the actual dose than were estimates derived from the clinical algorithm for 60% of the patients and were closer than the fixed dose for 695 of the patients.

In the light of this data, in 2007 the U.S. Food and Drug Administration updated warfarin labels to warn of sensitivity associated with these two genes and approved a commercially available generic test to identify variants contained within them.

Beta adrenergic antagonists and acute myocardial infarction

Anaesthesiologists routinely use beta adrenergic receptor antagonists to ensure intraoperative haemodynamic stability and improve outcome. It is well recognized that administration of beta adrenergic antagonists after acute myocardial infarction decreases mortality. In the midst of such standardization one group of investigators dared to ask a heretical question. Could there be heterogeneity of response with strong reduction of mortality in one group tempered by worse outcomes in another. To examine this question, the investigators enrolled 10 911 consecutive patients admitted to two Kansas hospitals in a study where they collected blood in order to examine beta adrenergic receptor genetic variability.

Previous data support an association between polymorphism of the beta-1 and beta-2 adrenergic receptors (ADRB1 and ADRB2) and surrogate end points of response to beta adrenergic blocker therapy.

In the study 735 total patients had an acute coronary syndrome, with 597 given a beta adrenergic receptor antagonist upon discharge. The study was to evaluate the effect of ADRB1 Arg 389Gly (1165 CG), Ser 49Gly 145 AG and ADRB2 Gly 16Arg (46 GA), Gln 72Glu (79 CG). The outcome was a multivariable-adjusted time to all cause 3year mortality.

There were 84 deaths during follow up. Four common beta1 and beta2 adrenergic receptor single nucleotide polymorphism were examined. Increases in mortality were found with possession of certain variants in the beta2 rising to 20% at three years with combination of Glu27Glu and Arg16Gly.

Mechanistically it appears from this study that patient with variants impairing beta2 adrenergic down regulation a situation where receptor function does not dampen benefit from beta adrenergic receptor blockers. However those with genotypes enhancing down regulation less receptor is present at the cell surface which mimics beta adrenergic receptor antagonist activity do not benefit. The administration of beta adrenergic receptor antagonists to such patients appears to unmask negative effects. Pending replication this study provides compelling evidence that genetic variability in the beta2 adrenergic receptor has direct clinical relevance.

Beta-agonists and asthma

Asthma is a preoperative diagnosis of concern to anaesthesiologists and bronchodilators prescribed to prevent or to treat airway hyperactivity also interact with beta2 adrenergic receptors. Recent evidence suggests that individuals with mild asthma who are homozygous for 16 Gly demonstrate long term improvement with either albuterol or long-acting beta2 agonists, where as those homozygous

for Arg 16 do not. In fact the latter patients appear to suffer adverse effects when regularly treated with short acting beta-agonists.

This phenomenon may well be to the same regulatory mechanism described above and reinforces the paradigm that patient's with enhanced beta2 adrenergic receptor down regulation may not benefit from beta2 adrenergic receptor antagonists for myocardial protection or beta2 adrenergic receptor agonists for bronchodilation. Instead alternative strategies may help this group of patients and the current concept of pharmacogenetics will impact the clinical management of asthma in the near future.

Intraoperative pharmacogenomic issues

The main conditions that an anaesthetist may have to deal with intraoperatively would include scoline apnoea, MH and porphyria. These conditions have already been described earlier in the presentation in terms of how they made an impact in the area of pharmacogenetics.

Volatile anaesthesia

An interesting example of differential general anaesthetic response was first documented by clinicians after astute observation. At the University of Louisville, in Kentucky, they recognized anecdotally that high concentrations of volatile anaesthesia were required for red headed patients, whose hair colour results from dysfunction in the melanocortin receptor.

After showing that anaesthetic requirements were increased in MCIR knockout mice, they tested desflurane requirements for females with red hair versus those with dark hair.

A subsequent study showed that red heads were also more sensitive to cold stimuli and had lower pain threshold after subcutaneous lidocaine. While a bit away from proving that MCIR is the cause of these differences, their work is bolstered by research that showed that female mice and human with MCIR mutations responded differently to k-opioid agonists.

Mogil and colleagues demonstrated that women with variant MCIR alleles demonstrated sensitivity to the k-opioid agonist pentazocine. Individuals with pale skin and red hair are more likely to carry inactivating variants of the MCIR gene and demonstrate reduced sensitivity to noxious stimuli and increased analgesic response to morphine-6-glucuronide.

Opioids for labour analgesia

The μ -opioid receptor encoded by the gene OPRM1, is the primary site of action of many endogenous opioid peptides, and is the major target of opioid analgesics. An adenine to guanine substitution at position 118 in gene OPRM1 has been reported to be common up to 30% of Caucasians carrying at least one G118 variant allele and the presence of at least one G118 allele has been associated with increased binding affinity and potency of beta-endorphins.

Dr Landau recently demonstrated a significant increase in sensitivity to the analgesic effect of intra thecal fentanyl in labouring women carrying the G118 allele of OPRM1; the converse is that women 118A homozygous required significantly more fentanyl for labour analgesia. The finding of a 1.5 to two fold difference in ED50 according to genotype is clinically relevant because provision of labour analgesia remains an ongoing challenge with the need to reduce doses and minimize opioid related side effects. This was the first published clinical trial demonstrating that genetic variants of μ -opioid receptors affect the response to neuraxial fentanyl or any other neuraxial opioid. If confirmed in other clinical settings and with other opioids, use of OPRM1 118A/G genotyping may improve the provision of analgesia in the not so distant future.

POST OPERATIVE ISSUES

Individual variation in pain perception and drug sensitivity can yield unpredictable results in opioid efficacy and side effects and tolerance profiles. Many candidate genes have been considered as suitable targets to study the genetic basis for this variable pain and analgesia experience. While some of these pharmacodynamic effects of OPRM1 genotypes were reported above, we now turn to genetic differences in an important drug metabolizing enzyme which impacts drug pharmacokinetics. CYP2D6 is perhaps the best studied of the hepatic enzymes, since it is involved with the metabolism of more than 100 drugs.

It influences the pharmacokinetics of many drugs used routinely in our practice including opioids, beta-blockers, antiarrhythmics, antipsychotics, tricyclic antidepressants, serotonin reuptake inhibitors and anti-emetic 5HT3 antagonists.

The CYP2D6 gene is highly polymorphic, with more than 75 different alleles, resulting in relative enzyme activity ranging from 1% to 200%. As a result an individual's CYP2D6 metabolism can be classified as being ultra-rapid, extensive, normal, poor or even absent (approximately 7% to 10% of Caucasians).

The prevalence of variant alleles exhibits considerable inter-ethnic differences for example the frequency of CYP2D6 gene duplication associated with ultra rapid metabolizers ranges from 0.5% in China to 29% in Ethiopia. Codeine is a prodrug whose action is determined by demethylation to morphine a reaction catalyzed by the enzyme CYP2D6. A number of in vivo studies have demonstrated detectable differences in plasma morphine concentration between extensive and poor metabolizers.

Yue and colleagues found that more than 5 times less morphine metabolites were excreted in poor metabolizers compared with extensive metabolizers after 25mg of codeine. Ultra-rapid metabolizers given 30mg of codeine demonstrated 50% larger concentration of morphine in the plasma compared with their extensive metabolizer counter parts in a study by Kirchheiner and colleagues.

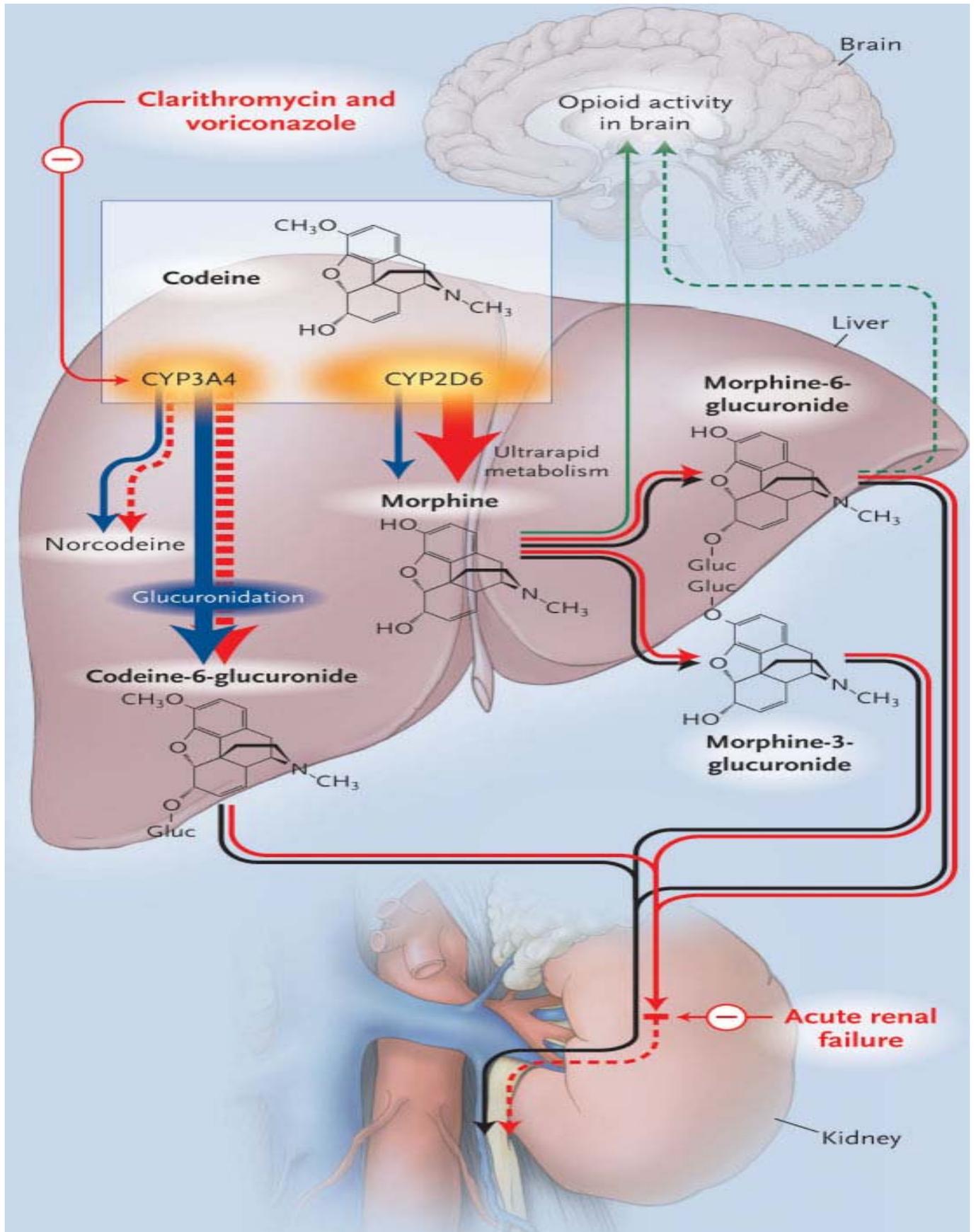
However clinical investigation of CYP2D6 genotypes in the post operative pain setting have shown conflicting results, and well designed prospective studies are lacking. Essentially patients who have poor metabolism do not achieve analgesia with codeine, while they still encounter side effects such as nausea and vomiting. Conversely codeine intoxication can be anticipated with ultra rapid CYP2D6 metabolism.

In an observation made by Yvan et al life threatening intoxication developed in a patient after he was given small doses of codeine for the treatment of cough associated with pneumonia. Codeine is bio-activated by CYP2D6 into morphine, which then undergoes further glucuronidation. CYP2D6 genotyping showed that the patient had three or more functional alleles, a finding consistent with ultra rapid metabolism of codeine. The intoxication was attributed to this genotype, in combination with inhibition of CYP3A4 activity by other medications and a transient reduction in renal function.

In 2007 the US FDA published a warning on codeine use in nursing mothers after the death of a breast fed 13 day old neonate believed to have suffered a morphine overdose because his mother was taking codeine and was a CYP2D6 ultra rapid metabolizer.

Metabolic Pathways of Codeine Biotransformation

Fig. 4



In conclusion 5% to 10% of Caucasians patient poor metabolizer are unlikely to gain full benefit from codeine administration but are just as likely to suffer codeine related side effects, although these studies are yet to be confirmed in post operative pain studies.

Tramadol exerts analgesic effects via the opioid metabolite M1 O-demethyltramadol and via modulation of noradrenergic and serotonergic monoamine pathway. O-demethylation of tramadol to the opioid agonist M1 is mediated by CYP2D6 and laboratory studies have shown significantly lower plasma concentration of M1 in poor metabolizers compared to extensive metabolizer genotypes and subsequent reduced analgesic effects in experimental pain.

In a prospective study Stamer and colleagues studied 300 patients given tramadol after abdominal surgery. Poor metabolizers were twice as likely to be non-responders and received twice the rescue analgesia. Tramadol consumption was 30% higher in the poor metabolizers group compared to with the extensive metabolizer group. Subsequent studies have confirmed the high non-response rate and increased dose requirements in patients with a poor metabolizer genotype.

In another study it was observed that patients with renal impairment while at the same time being ultra rapid metabolizers of tramadol were predisposed to respiratory depression. Analysis of the patient's genotype revealed that they had a CYP2D6 duplication resulting in ultra rapid metabolism of tramadol to its active metabolites +O-demethyltramadol. Concomitant renal impairment resulting in the decreased metabolite clearance resulting in enhanced opioid toxicity. This genetic CYP2D6 variant is particularly common in specific ethnic groups and should be a future diagnostic target whenever administration of tramadol or codeine is anticipated as both drugs are subject to comparable CYP2D6 dependent metabolism.

In one study some investigators sought to answer the question as to whether CYP2D6 copy and polymorphisms affect the success or failure of ondansetron prophylaxis. Some patients treated with ondansetron for PONV do not respond to therapy. One possible mechanism for this failure is ultra rapid drug metabolism via the cytochromeP450 system, specifically the enzyme CYP2D6.

The study was designed to determine whether prophylactic ondansetron and had multiple copies of CYP2D6 allele had an increased rate of PONV. A group of 250 female patients undergoing standardized general anaesthesia were given 4mg of ondansetron 30min before extubation.

Patients were observed for symptoms of nausea and vomiting. DNA was extracted from blood in all patients and was analyzed by using a gene specific

probe to determine the CYP2D6 gene copy number and genotyped by PCR amplification with a custom oligonucleotide micro assay to determine the specific CYP2D6 genotype.

The results of the study were that 88 patients experienced nausea and 37 of those also had vomiting. When analyzed by genotype the incidence of vomiting in poor, intermediate, extensive and ultra rapid metabolizer were 8%, 17%, 15%, and 45% respectively. p value < 0.001 vs all other groups.

The conclusion is that patients with three copies of CYP2D6, a genotype consistent with ultra rapid metabolism or both have an increased incidence of ondansetron failure for the prevention of post operative vomiting but not nausea.

CONCLUSION

Despite its gains in other areas of medicine for example psychiatry and oncology, pharmacogenomics has had limited impact in the clinical practice of anaesthesiology. The impetus of genotyping a patient in clinical practice is that it needs to have an impact on the outcome or the drug being evaluated has to be costly and/or difficult to predict clinically. Although a negative outcome such as PONV is relatively costly it does not currently compare to the cost, time and effort required to genotype a patient to possibly lower the incidence of such an event. However it is conceivable that in the not too distant future, patients undergoing anaesthesia will have as part of their pre operative screening genetic profiling to detect life threatening risk factors.

Ultimately a secured online data base could be produced with each individual's genotype available to the care provider. Such a system would raise important ethical and social questions, but is potentially of major clinical and economic importance.

GLOSSARY

Allele; two or more alternative forms of a gene occupying the same position / locus on the chromosome. For example the gene for eye colour has various forms (alleles) brown and blue alleles

Genome: the human genome consists of 23 pairs of chromosomes, which are collectively referred to as the karyotype. There are 22 pairs of autosomes and 1 pair of sex chromosomes XX or XY

Gene: a hereditary coding unit comprising of a specific DNA sequence occupying a specific position / locus on the chromosome

Locus: the specific position a gene occupies on a chromosome

Autosome: a non-sex chromosome (22 pairs in human)

SNP: single nucleotide polymorphism (pronounced snip). The commonest type of human genetic or allelic variation. Occurs frequently $> 1\%$ within the general population.

Phenotype: outward physical appearance e.g. blue-eyed person

Genotype: genetic make up that determines the phenotype e.g. genes for blue eyes is the genotype; this determines the phenotype a blue-eyed person.

Genetic linkage: alleles situated close together on the same chromosome tend to be inherited together.

Haplotype: a combination of closely linked alleles on a single chromosome that are inherited together.

Mutation: a variation that occurs in $< 1\%$ of the population.

Polymorphism: a variation that occurs in $> 1\%$ of the population.

Knock out mice: strain of mice in which an endogenous gene has been removed bred out

Knock in mice: strain of mouse in which an endogenous gene has been replaced with an altered copy.

Transgenic mice: strain in which a gene, not normally present, has been introduced.

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