Pharmacogenetics and anaesthetic drugs: Implications for perioperative practice

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Abstract
Pharmacogenetics seeks to elucidate the variations in individual's genetic sequences in order to better understand the differences seen in pharmacokinetics, drug metabolism, and efficacy between patients. This area of research is rapidly accelerating, aided by the use of novel and more economical molecular technologies. A substantial evidence base is being generated with the hopes that in the future it may be used to generate personalised treatment regimens in order to improve patient comfort and safety and reduce incidences of morbidity and mortality. Anaesthetics is an area of particular interest in this field, with previous research leading to better informed practice, specifically with regards to pseudocholinesterase deficiency and malignant hyperthermia. In this review, recent pharmacogenetic data pertaining to anaesthetic drugs will be presented and possible future applications and implications for practice will be discussed.

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1. Introduction

The publication of the human genome in its entirety in 2003 signalled a new dawn in the quest for a greater and more complete understanding of the variations which govern genes and the proteins which they express. Much of the promise and potentials of the human genome project remains to be developed and seen, however, as medical science enters the post-genomic era, accompanied by ever greater advances in rapid DNA sequencing technologies, it is anticipated that the knowledge gained from the human genome project will be instrumental in advancing developments in pharmacogenetics and personalised medicine [1].

Pharmacogenetics and pharmacogenomics are terms which are often used interchangeably in medical literature, however, they are distinct entities [2]. Pharmacogenetics refers to the study of variability in an individual’s response to a drug due to heritable factors [3]. Much of the research in this field has been evaluating the association of single nucleotide polymorphisms with how individuals metabolise drugs. Pharmacogenomics is a more recent, broader term which may be regarded as the application of pharmacogenetics to the whole genome and across populations [4], encompassing proposed outcomes such as generating drug–response profiles unique to each individual based on their genetic make-up [5], examining the effect of drugs on gene expression [6,7], and the eventual utilisation of genomic principles in the development and trialling of new drugs [8] (See Table 1).

2. Epidemiology of perioperative care and complications

Globally, it is estimated that 234 million surgical procedures are carried out annually. It is thought that 7 million patients experience harm, and 1 million die each year post-operatively worldwide [9]. It is furthermore estimated that up to 50% of this harm is avoidable [10]. The 2012 European Surgical Outcomes Survey examined mortality rates across 28 European countries and found that in the UK cohort there was a 3.6% mortality rate following non-cardiac procedures. The 2012 European Surgical Outcomes Survey examined mortality rates across 28 European countries and found that in the UK cohort there was a 3.6% mortality rate following non-cardiac procedures. The 2012 European Surgical Outcomes Survey examined mortality rates across 28 European countries and found that in the UK cohort there was a 3.6% mortality rate following non-cardiac procedures. The 2012 European Surgical Outcomes Survey examined mortality rates across 28 European countries and found that in the UK cohort there was a 3.6% mortality rate following non-cardiac procedures.

It should be noted that surgical mortality rates have fallen in recent decades, and this is furthered by a host of different factors including age, sex, disease states, and epigenetic factors [18]. CYP enzyme genes and their alleles may be affected by a variety of different mutations of which 2000 have been described [19]. Such polymorphisms include frameshift mutations, deletions, and splicing defects, all of which may affect both introns and exons, including at promoter regions of the gene, thereby having the potential to affect gene transcription. The functional effect of such polymorphisms is an increase or decrease in the activity of the gene (and thereby its enzyme) [20].

Based on the variation of functionality of proteins due to genetic polymorphisms, it is possible to classify individuals into different phenotypic classes based on enzymatic activity: poor metabolisers who demonstrate no CYP activity, intermediate metabolisers who demonstrate reduced activity, extensive metabolisers who demonstrate normal activity, and ultrarapid metabolisers who demonstrate increased activity [21]. By using this system, much of the variation in patient responses to certain opiates commonly used in anaesthetic practice e.g. codeine, tramadol, oxycodone is explained. These drugs are normally metabolised in their active, effective form by CYP2D6. However, poor metabolisers who carry two non-functional alleles for the CYP2D6 gene will experience little analgesic effect owing to a non-functional CYP enzyme [22], whilst ultrarapid metabolisers who have three functional allele copies experience higher plasma concentrations of morphine due to CYP hyperactivity and are more prone to opiate toxicity [23]. This is one example of how existing knowledge of pharmacogenetic differences between individuals has had an impact on knowledge of pharmacokinetics and drug efficacy.

3. The molecular basis of interindividual variation in pharmacogenetics

The elimination of a drug from the body involves two processes: metabolism and excretion. Metabolism occurs in two stages: catabolic phase I reactions (e.g. hydrolysis, oxidation) and anabolic phase II reactions (e.g. addition of a glucuronyl or methyl group to the metabolite to polarise it). Excretion primarily occurs via the kidneys, hepatobiliary system, or lungs.

The cytochrome P450 (CYP) superfamily of enzymes is of particular interest when considering phase I reactions. There are 59 P450 proteins which are categorised into 18 families and 43 sub-families, the majority of which are expressed in the smooth endoplasmic reticulum of hepatocytes, with CYP1, CYP2 and CYP3 being the main families involved in human drug metabolism. Up to 80% of drug metabolism is performed by these three families of enzymes [17]. The expression, and therefore functionality of CYP proteins is influenced by a host of different factors including age, sex, disease states, and epigenetic factors [18]. CYP enzyme genes and their alleles may be affected by a variety of different mutations of which 2000 have been described [19]. Such polymorphisms include frameshift mutations, deletions, and splicing defects, all of which may affect both introns and exons, including at promoter regions of the gene, thereby having the potential to affect gene transcription. The functional effect of such polymorphisms is an increase or decrease in the activity of the gene (and thereby its enzyme) [20].

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| Table 1 |
|---------|
| Gene | A Section of DNA which codes for a protein |
| Genome | The entire genetic material of an organism |
| Nucleotide | A basic unit of DNA comprising an organic base, ribose sugar, and negatively charged phosphate group |
| Mutation | A change to the DNA sequence of an organism |
| Variation | Differences seen between members of the same species |
| Genotype | The genetic make-up of an organism |
| Phenotype | The physical appearance of an organism arising from its genotype |
| Polymorphism | A gene which has more than one allele allowing for phenotypic variation in a population |
| Single nucleotide polymorphism | Variation of a specific nucleotide seen in >1% of a population |
| Haplotype | A set of genetic variations which are inherited together |
In addition to mutations to enzymes which are involved in drug elimination, another aberration which has the potential to affect human drug metabolism is mutations to transport proteins. The ATP binding cassette (ABC) family of transport proteins comprises 50 different members in humans which have been the subject of much research owing to their ubiquity, being highly expressed at xenobiotic compounds such as intestinal enterocytes, hepatocytes and the renal epithelium [24]. They are also expressed in brain capillary endothelial cells forming the blood–brain barrier.

ABCB1 (also known as P-glycoprotein and MDR1) is the most prominent example of an ABC protein. It undergoes ATP binding and subsequent hydrolysis, propagating the efflux of molecules from the cell. The ABCB1 gene is susceptible to single nucleotide polymorphisms which may be inherited individually or as a haplotype. In 2014, He et al. conducted a study which concluded that individuals with two of these variants, the CG haplotype (C3435T and G2677T) were more likely to experience chemotherapy induced nausea and vomiting despite receiving ondansetron. The authors suggested that as the CG haplotype was associated with greater ABCB1 expression, those individuals might have a decrease in levels of CNS accumulation of ondansetron [25], showing that variations in the genotype of transport proteins have the potential to affect drug function and patient response [26].

Drug receptors themselves also represent sources of polymorphisms which have an effect on the efficacy and pharmacodynamics properties of their drug ligands. The β2 adrenergic receptor (also known as ADRB2) gene is located at chromosome 5q31-32 and at least nine polymorphisms have been identified at its coding region [27]. In 2006, Smiley et al. investigated hypotension in the context of obstetric spinal anaesthesia and found that women who were homozygotes for the Gly16 or Glu27 polymorphisms required lower dosages of ephedrine to raise their systolic blood pressure [28]. In 2015, the same authors this time investigated the role of phenylephrine in counteracting hypotension seen following spinal anaesthesia for Caesarean section and found that women homozygous for Gly16 in the ADRB2 gene also required a lower phenylephrine dose to regain adequate systolic blood pressure control (though the effect was lesser than that seen with ephedrine) [29].

Existing research has elucidated that genetic and molecular variations in drug enzymes, transport proteins and receptors have the potential to greatly affect a given drug’s metabolism, rate of uptake, excretion, and ultimately its efficacy and potential for toxicity. These molecular variations represent attractive targets for future pharmacogenetic investigation and research.

4. Pharmacogenomics as applied to perioperative medicine

Implications for perioperative care

The data in Tables 2 and 3 lists a selection of genetic variants in anaesthetic drugs which contribute to the differences seen in efficacy, pharmacokinetics, and side effects which patients may experience during the perioperative period. The data generated

### Table 2
Pharmacogenetics of anaesthetic agents.

| Drug                 | Description                                      | Gene(s) affected by polymorphism | Genetic variant | Phenotypic effect of polymorphism                                      |
|----------------------|--------------------------------------------------|----------------------------------|-----------------|------------------------------------------------------------------------|
| Propofol             | IV induction agent potentiating the inhibitory action of GABA at GABA<sub>A</sub> receptors | UGT1A9                          | 1887 T/G        | Higher induction dose required [30]                                     |
|                     |                                                  |                                  | 331C/T          | Higher levels of drug clearance [31]                                   |
|                     |                                                  |                                  | 1818T/C         | Longer time needed for loss of consciousness                           |
|                     |                                                  |                                  | CYP2C9          | Higher plasma concentration [32]                                       |
| Isoflurane           | Volatile agent used for maintenance, acts to potentiating GABA via GABA<sub>A</sub> receptors | RYR1                            | Tyrosine 522     | Malignant hyperthermia [33]                                            |
| Sevoflurane          | Volatile agent used for induction and maintenance, acts to potentiating GABA via GABA<sub>A</sub> receptors and inhibition of transmission at NDMA receptors | CYP2E1                          | Variations in levels of enzyme expression                              |
|                      |                                                  |                                  | Gly2130Arg       | Renal dysfunction [34]                                                 |
|                      |                                                  |                                  | CYP2B6           | Decreased drug clearance [35]                                          |
| Ketamine             | IV anaesthetic used in shocked patients and children, acts as an NM2A receptor antagonist | SCN9A                           | Reduced elimination, increased duration of action and recovery time [36] |
|                      |                                                  |                                  | 395N > K         | Reduced analgesic efficacy [37]                                         |
| Lidocaine            | Local anaesthetic acting via the blockage of sodium channels | MCR1                            | Reduced hydrolysis, increased duration of action leading to prolonged neuromuscular blockade and apnoea [38] |
|                      |                                                  |                                  |                 |                                                                        |

### Table 3
Pharmacogenetics of other perioperative medications.

| Drug                 | Description                                      | Gene(s) affected by polymorphism | Genetic variant | Phenotypic effect of polymorphism                                      |
|----------------------|--------------------------------------------------|----------------------------------|-----------------|------------------------------------------------------------------------|
| Fentanyl             | μ-opioid receptor agonist inhibiting neurotransmission | OPRM1                           | 304 A/G         | Variations in median effective dose required to achieve analgesic effect [39] |
| Suxamethonium        | Depolarising neuromuscular blocker                | BChE                             | 293A > G        | Reduced hydrolysis, increased duration of action leading to prolonged neuromuscular blockade and apnoea [39] |
|                      |                                                  |                                  | 1699G > A       |                                                                         |
|                      |                                                  |                                  | 695T > A        |                                                                         |
|                      |                                                  |                                  | RYR1            | Malignant hyperthermia [40]                                            |
|                      |                                                  |                                  | Multiple at     |                                                                         |
|                      |                                                  |                                  | 19q13.1          |                                                                         |
|                      |                                                  |                                  | c520C > T       |                                                                         |
|                      |                                                  |                                  | rs2306283 A > G |                                                                         |
|                      |                                                  |                                  | rs11288303 C > T|                                                                         |
| Rocuronium           | Non-depolarising neuromuscular blocker            | SACNA15                         | 2677 TT         | Increased bioavailability, reduction in PONV [41]                       |
|                      | Anti-emetic 5-HT3 receptor antagonist              | SLC01B1                         | 3435 TT         |                                                                         |
|                      |                                                  | ABCB1                           |                 |                                                                         |
|                      |                                                  | ABCB1                           |                 |                                                                         |
| Ondansetron          |                                                  |                                 |                 |                                                                         |
from the mining of genetic sequences and variants has the potential to be applied to a number of different perioperative drugs and anaesthesia related conditions which are discussed below.

One example of how drug interactions based on genetic variations can affect clinical practice is the management of malignant hyperthermia (MH). MH is an autosomal dominant condition characterised by hypermetabolism, hypoxia, hypercapnia and hyperthermia resulting from abnormal calcium homeostasis. This is thought to be due to mutations in the ryanodine receptor gene (RYR1) in 70% of cases. Mutations to the CACNA1S gene are thought to be the cause in 1% of the population [44].

MH is triggered by volatile anaesthetics or suxamethonium (detailed in Table 2), so for this reason dantrolene (a skeletal muscle relaxant which inhibits calcium release from the sarcoplasmic reticulum) is now kept in theatres and recovery areas. All patients suspected of having experienced MH undergo a muscle biopsy which undergoes contracture testing by being exposed to halothane and caffeine: contraction is a positive result. Patients whose MH status is positive or equivocal can then receive medic alert bracelets and should receive MH-safe anaesthesia e.g. total intravenous anaesthesia (TIVA) in place of gaseous anaesthesia in subsequent operations. The MH mortality rate has fallen from a peak of 80%–5% [45] due to the supportive measures detailed above, particularly the use of dantrolene and effective testing.

One condition which may represent a future target for pharmacogenomic research as applied to anaesthetics is the propofol infusion syndrome (PRIS), a potentially fatal condition characterised by metabolic acidosis, rhabdomyolysis, and arrhythmias which occurs following prolonged propofol administration (>48 h) at high doses (>4 mg/kg/h) [46]. The precise aetiology of this condition is unknown, but a case report by Karakitsos et al. (2007) suggested that it may have a genetic basis and that patients who have experienced PRIS should undergo genotyping so that genetic screening might be developed [47]. If such screening is successfully developed on the basis of current and future pharmacogenomic data - with high specificity - patients could be appropriately selected for TIVA, and the incidence of PRIS could be reduced.

The prospect of generating genetic profiles which allow an individual's treatment regimen to be personalised to their specific pharmacokinetic genotype is a deeply exciting one. Individuals could be screened in order to determine their metabolic status when it comes to speed of elimination of opiate analgesics. This could lead to an improvement in therapeutic efficacy and a reduction in cases of drug toxicity, especially for medications which have a narrow therapeutic window. For example poor CYP metabolisers could be more carefully monitored for signs of toxicity or receive non-opiate analgesia if appropriate. Similarly, those with opiate receptor polymorphisms (as with fentanyl, presented in Table 3) could receive accurate drug dosages titrated to their personal capacity for drug metabolism and rate of elimination. This could lead to a reduction in the incidence of perioperative morbidity and mortality.

The recent data published in Mei et al.'s study (2015) and presented in Table 3 suggests that rocuronium's pharmacokinetics and efficacy may also be influenced by pharmacogenetic factors with a longer duration of action observed with SLCO1B1 and ABCB1 gene variants. A blood test already exists for BChE (pseudocholinesterase) deficiency which utilises dibucaine to indicate if an individual is homozygotic for the mutant alleles and thereby likely to be susceptible to prolonged blockade and apnoea in the event of suxamethonium administration; further studies into the pharmacogenomics of rocuronium might mean that similar tests could also be developed for the non-depolarising neuromuscular blockers such as rocuronium.

5. Concluding remarks

The cost of sequencing an individual's DNA in the clinical setting in 2015 is estimated to be approximately $3000 and declining [48]. As the prospect of sequencing a genome for $1000 or less gradually becomes feasible, it is hoped that data mining efforts can be redblue. Whilst this has immense potential for breakthroughs in identifying novel polymorphisms, the ethical implications of pharmacogenomic research will also need to be considered, not least in determining the most optimum way of storing patient data in a safe yet accessible manner.

In conclusion, as the cost of gene sequencing technology falls and the number of genome wide association studies evaluating the effect of genetic polymorphisms on drug responses rises, there is potential that the evidence base generated from pharmacogenomic data can be translated into appropriate clinical guidelines which will complement existing anaesthetic considerations in order to deliver the best possible care to patients based on their unique genetic variations, thereby improving perioperative care and maximising patient outcomes.

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