Pharmacogenomic variability and anaesthesia

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The concept of ‘personalized medicine’ in which a knowledge of genetic factors guides prescribing tailored to the individual is popularly considered to be an inevitable consequence of completion of the International Human Genome Project. We should not forget, however, that a personal or family history of one of several uncommon pharmacogenetic conditions has influenced the use of the implicated drug(s) during anaesthesia for the past 50 yr. Although this has been important for those affected, pharmacogenomics heralds the prospect of an individual’s genetic profile informing every prescription. Progress has been rapid in some areas, notably cancer chemotherapy where response to treatment can be predicted on the basis of the genetic profile of the tumour cells. The situation is different for most currently available drugs, including those used by anaesthetists, where genetic variability to drug response is presumed to be the result of a complex interaction of multiple factors. We review the nature and investigation of pharmacogenomic variability and contrast the progress made with research into opioid variability with the more limited literature concerning i.v. and inhalation anaesthetics.

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Genetic factors have been recognized to influence individual patient responses to drugs used in anaesthesia for more than 50 yr. Indeed, the first review of pharmacogenetics and anaesthesia appeared in the same issue of Anesthesiology as the description of the second gas effect, and Winnie’s description of the subclavian perivascular approach to brachial plexus anaesthesia. It is important, however, to appreciate the distinction between the subject matter of Kalow’s review and recent developments in the understanding of the genetic components underlying inter-individual variability in responses to drugs that have flowed from the revolution in molecular biology technology and the human genome project.

Kalow described a series of rare conditions that each appeared to follow a Mendelian pattern of inheritance and which predisposed the affected individual to an idiosyncratic response to a drug or group of drugs used during anaesthesia or in the perioperative period. The conditions included myotonic syndromes, pseudocholinesterase deficiency, porphyrias, and anaesthesia-induced hyperthermia, now known as malignant hyperthermia (MH). Perhaps, the best way to envisage the distinction between this pharmacogenetic variation and pharmacogenomics, which describes the genetic contribution to population variability in drug responses, is to consider pharmacogenomic variability as encompassing subgroups within an overall unimodal response whereas pharmacogenetic variability is so marked that it defines separate peaks in bimodal or even multimodal population responses (Fig. 1).

In general terms, genetic variants underlying pharmacogenetic disorders are rare and solely determine the abnormal drug-response phenotype, whereas genetic variants involved in pharmacogenomic variability are relatively common but make only a partial contribution to the drug-response phenotype. In other words, pharmacogenomics deals with the genetics of drug-response phenotypes that have a complex genetic basis. It is an understanding of this pharmacogenomic variability that underpins the concept of personalized medicine and which we will focus on in this review. It will first be necessary to describe the nature of genetic variants before discussing the pharmacological processes that they can influence. We will use the pharmacogenomics of opioids as an exemplar situation of the difficulties in developing the knowledge base required for personalized medicine. Finally, we will review the relatively limited evidence for pharmacogenomic variability of responses to i.v. and inhalation anaesthetics.
Some basic genetic principles

Chromosomes

The great majority of genetic information is contained within the chromosomes found in the cell nuclei (the remainder being in the mitochondria, inherited solely from the mother). The chromosomes are formed predominantly of coils of DNA, which is a double helix comprising a sugar backbone (deoxyribose) and purine and pyrimidine nucleotide bases. The double-stranded DNA can be envisaged as a coiled ladder in which the chain of deoxyribose molecules form the vertical supports and the rungs are formed by complementary pairs of bases. Of the four bases, adenine pairs with thymine and cytosine with guanine. It is the order of these four bases along the chromosome that forms the template defining the sequence of amino acids of the proteins produced in the cell.

Genes and proteins

Each protein produced in a cell is encoded by a specific DNA sequence found at a specific chromosomal locus and this DNA sequence is known as a gene. The Human Genome Project has revealed that there are 30 000–40 000 genes in the human genome, whereas Venter and colleagues estimated that 75% of the genome is composed of intergenic DNA: this DNA may have structural or regulatory functions or it may simply be redundant. Furthermore, not all of the sequence within a gene is ultimately translated into an amino acid sequence. The coding sequence of a gene is interspersed with non-coding sequence to form exons and introns, respectively (Fig. 2): exons constitute only ~5% of the DNA within a gene on average. The process of transcription, the DNA uncoils and single-stranded pre-messenger RNA (pre-mRNA) is formed by the addition in sequence of bases complementary to those of the DNA, that is, with cytosine being complementary to guanine but uridine (not thymine as occurs in DNA) being complementary to adenine. The initial transcript contains the complementary sequence to the exons and introns, but the intronic sequence is spliced out to form mRNA before it translocates to the cytoplasm where translation into the amino acid sequence takes place.

In addition to the introns, there is an additional sequence of non-coding DNA within the gene that lies upstream of the coding region. This non-coding DNA is called regulatory DNA and it plays a role in controlling the expression of the gene. The regulatory DNA can be further divided into two types: promoter and enhancers. Promoters are sequences that are located at the beginning of a gene and are responsible for initiating transcription. Enhancers, on the other hand, are sequences that can increase the level of transcription of a gene and are usually located at a distance from the gene they regulate.

Fig 1 Pharmacogenetic vs pharmacogenomic variability. Pharmacogenetic variability is used to describe idiosyncratic reactions in which a (usually) rare genetic variant has a major effect on the response to a drug such that carriers of the variant form a distinct population (red) in contrast to those without the variant (blue). Pharmacogenomic variability relates to the combination of genetic factors that each has a small contribution to population variability. Carriers of such a variant (green) form a subpopulation that has a response to the drug that tends towards one or other tail of the whole population. The relative sizes of the distribution curves are not to scale.

Fig 2 Gene structure and transcription. The DNA of the coding region is composed of exons (coding DNA) interspersed with introns (non-coding DNA) and is flanked by untranslated regions (UTRs). Upstream of the coding regions within the gene are DNA sequences that control (promoter) and regulate (enhancers) gene expression. During transcription, the initial nuclear transcript includes RNA sequence complementary to the entire coding region and the UTRs. In a subsequent step, the introns are spliced out to form mRNA which translocates to the cytoplasm.
‘upstream’ of the first exon. This DNA includes regions that are important in the determination of whether the gene is expressed in any given cell type and, if it is expressed, in the regulation of its expression.

During translation, the amino acids to be incorporated in the protein are determined by successive triplets of bases, or codons, in the mRNA sequence. The genetic code refers to the combination of bases within a codon that defines each amino acid. Some amino acids are encoded by more than one codon, which is possible because there are 64 codons and only 20 amino acids: there is also a codon that initiates translation and three codons that signal the termination of the polypeptide sequence (stop codons).

**Genetic variation**

In the terminology of the biologist, a mutation is any change in the sequence of bases in the genetic material of an organism: mutations in germ line cells have enabled evolution through natural selection. For a mutation to be retained in the gene pool, it must be non-deleterious to the likelihood of the ability of the carrier to reproduce. Between-species variation has occurred mainly through chromosomal mutations that can lead, for example, to deletion of one or more genes, duplication of genes, or generation of a new gene from the juxtapositioning of two genes previously separated by intergenic DNA.

Within-species genetic variation is more likely to arise from smaller scale mutation events affecting single genes and often a single base pair of the gene sequence. These may be insertion or deletion of a base pair or substitution of one base for another (point mutation). The consequences of a mutation affecting a single base pair can range from no effect to a major effect on the protein and this depends upon which bases are involved and their location within the gene. Examples of the range of effects of base pair substitutions in exonic DNA are illustrated in Figure 3, but mutations in non-coding regions of the gene can also have a biological effect. For example, an intronic mutation may affect the splicing of pre-mRNA resulting in exclusion of an exon from the mRNA or inclusion of intronic sequence in the mRNA. Another example is a mutation in the promoter region of the gene, which may affect gene expression.

The apparently infinite variation in the human form, in terms of physical and physiological characteristics, is largely attributable to the many possible combinations of base pair substitutions that have accumulated in the human gene pool through evolution of our species. Although these substitutions have arisen as mutations, in the context of human (especially clinical) genetics, the use of the term ‘mutation’ is restricted to uncommon (by convention, <1% prevalence) variants that are implicated as the cause of classical (single gene) genetic disorders. Other variants are known as single-nucleotide polymorphisms (SNPs).

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**Fig 3** Possible effects of single base pair mutations. A hypothetical wild-type mRNA transcript and corresponding amino acid sequence is illustrated in (A). In (b), deletion of a uridine base causes a frameshift resulting in an extensive missense mutation in the amino acid sequence. In (c), an insertion of a uridine base causes a frameshift resulting in a nonsense mutation and truncation of the protein. Base pair substitutions can lead to no change in amino acid sequence (synonymous substitution, D), a change of a single amino acid in the protein sequence (missense or non-synonymous substitution, E), or truncation of the protein (nonsense substitution, F).
than 3 million SNPs have been characterized in the human genome by the International HapMap consortium. SNPs may be synonymous (not leading to an amino acid change) or non-synonymous. Non-synonymous SNPs may or may not influence the phenotype: this depends on the functional significance of the amino acid position involved and, if this is important, the nature of the amino acid substitution (influenced, for example, by the size and charge of the amino acids). Increasingly sophisticated bioinformatic programs are becoming available for predicting whether amino acid substitutions are likely to lead to changes in function of the relevant protein.

It can, however, be extremely difficult to predict the relevance of an SNP. An SNP may lead to a relative, or even complete, loss of function of a protein, but this may result in up-regulated expression of an alternative protein with the same or similar function, thereby reducing the impact of the SNP. Similarly, an SNP that produces a gain of function in one protein may have the consequence of leading to up-regulation of a protein having the opposite effect. Non-synonymous SNPs are increasingly recognized, however, as the hereditary factors in complex genetic disorders, such as diabetes, ischaemic heart disease, and rheumatoid arthritis. In a complex genetic disorder, multiple gene products interact to determine the phenotype, which may be the disease itself or the risk of developing the disease depending on environmental factors, such as diet, smoking, viral exposure, etc. Whether each of the gene products that may contribute to the phenotype does so in any individual will depend on the combination of SNPs (the haplotype) carried by that individual in the relevant gene; haplotypes may be associated with added risk, no risk, or they may be protective.

**Pharmacogenomic variability as a complex genetic trait**

The clinically relevant phenotype for pharmacological variability is the response of the individual patient to a drug. Pharmacodynamic variability (in therapeutic or adverse effects) describes differences in response to equal effect-site concentrations, whereas pharmacokinetic variability defines how soon after administration of a drug a therapeutic (or adverse response) effect-site concentration is reached and for how long it is maintained. The sources of pharmacokinetic variability are many and these are illustrated in Figure 4, but it is often not appreciated that multiple factors also potentially influence pharmacodynamic variability (Fig. 5).

**Investigation of pharmacogenomic variability**

The basis of identifying genetic determinants of drug response is the exploration of the association between a drug-response phenotype and a genetic variant. Knowledge of large numbers of SNPs spread across the genome and viable costs of genotyping (through availability of SNP array chips containing 500,000–1 million SNPs) has brought to reality the prospect of genome-wide association studies for several important diseases with a complex genetic background such as coronary artery disease, bipolar disorder, Crohn’s disease, rheumatoid arthritis, and diabetes. Genome-wide association studies benefit from having no requirement to identify prospective candidate genes or SNPs at the outset, but because of the extent of multiple comparisons, the numbers of cases and controls (in the order of 2000 each) need to be large for the stringent significance levels (typically \( P < 5 \times 10^{-7} \)) required to limit the false-positive rate.

To date, no sufficiently large collections of DNA samples of patients with altered anaesthetic drug phenotype have been entered into a genome-wide study. Rather, the approach has been to investigate the association of drug response with a specific SNP in a gene that could plausibly influence the phenotype (candidate gene), based on current knowledge of the gene product. These are invariably case–control studies, where the controls carry the usual allele and the cases the variant allele. The data are presented as the result of a \( \chi^2 \) test of association or
preferably as an odds ratio, with confidence interval, for the genetic variant to be associated with the less common phenotype. Several authors (e.g. Lotsch) have emphasized the need to check for genotyping errors and sampling bias (especially problematic with selection of controls) by demonstration of compatibility of the data with Hardy–Weinberg equilibrium. Furthermore, Cordell and Clayton have detailed other problems with this approach: they remark on the paucity of replication studies that confirm the findings of an original small case–control association study.

The pharmacogenomics of opioids

Opioids have been widely used within the fields of anaesthesia and acute and chronic pain for many years, and their use is characterized by large inter-patient variations in dosage requirements. Although many factors can influence pain perception and sensitivity, some of the variable clinical response to opioids can be explained by genetic heterogeneity in factors affecting both the pharmacodynamic and the pharmacokinetic behaviour of these drugs.

Pharmacodynamics

$\mu$-Opioid receptor

Genetic influences on the pharmacodynamic effects of opioids centre on variations in opioid-mediated targets, such as the $\mu$-opioid receptor, and factors influencing neurotransmitter pathways, such as catechol-$O$-methyltransferase (COMT). The $\mu$-opioid receptor acts via inhibitory G-proteins to initiate a cascade of complex neuromodulatory processes resulting in analgesia and remains the principal binding site for opioid drugs. Genetic polymorphisms resulting in changes to $\mu$-opioid receptor density and function help to explain variations in inter-patient opioid effects.

Many variants of the $\mu$-opioid receptor gene OPRM1 have been documented; however, one of the most commonly identified SNPs is c.118A>G (A118G in alternative nomenclature) with an allele frequency of 2–40% depending on ethnic population. Bond and colleagues suggested that this SNP results in changes to the extracellular binding site of the $\mu$-opioid receptor which exhibits subsequent altered binding with endogenous $\beta$-endorphins and exogenous opioid drugs. Subsequent research has also demonstrated altered signal transduction pathways in carriers of the c.118A>G allele. Laboratory studies in healthy volunteers have investigated the effect of the c.118A>G genetic polymorphism; Oertel and colleagues demonstrated that homozygous carriers of the c.118A>G gene required two to four times larger alfentanil concentrations to produce the same analgesia in response to electrically and chemically induced pain compared with wild-type genetic variants. The same study demonstrated that 10–12 times higher alfentanil concentrations were needed to produce the same degree of respiratory depression in the c.118A>G variant group. Carriers of the c.118A>G variant allele require lower doses of levomethadone and morphine-6-glucuronide to produce miosis compared with non-carriers.

Clinical studies investigating the effect of c.118A>G polymorphism in the surgical population tend to show a trend towards increased opioid requirements in keeping with laboratory findings. Chou and colleagues demonstrated increased morphine requirements in patients homozygous for the c.118A>G variant in the first 24 h after total abdominal hysterectomy, but no statistically significant difference in morphine requirements after 48 h. In a similar study investigating morphine requirements after total knee arthroplasty, c.118A>G homozygous patients required significantly more morphine in the first 24 h after...
surgery [40.4 (22) mg] compared with heterozygous AG and AA patients [25.3 (15.5) and 25.6 (11.7) mg, respectively].

Both studies have, however, been criticized for failing to demonstrate random genetic sample populations in keeping with the Hardy–Weinberg principle. Hayashida and colleagues studied 138 Japanese patients undergoing major abdominal surgery and recorded total opioid consumption (including epidural and rescue opioids) for 24 h after surgery. Homozygous c.118A>G carriers required significantly more opioid analgesia compared with heterozygous or homozygous wild-type carriers. Although all patients in this study received epidural fentanyl or morphine, a variety of rescue opioids and routes of administration were used, making comparisons between individuals difficult. In contrast, patients who are homozygous for the c.118A>G allele may be more sensitive to intrathecal opioids than their heterozygous or wild-type counterparts. In a recent study, Landau and colleagues demonstrated lower intrathecal fentanyl requirements in labouring women homozygous for the c.118A>G allele.

In a pilot study, Coulbault and colleagues failed to find a significant association between the c.118A>G polymorphism and postoperative morphine requirements in patients undergoing colorectal surgery, although there was a trend for larger doses in those with the G variant allele. Janicki and colleagues failed to find a significant association between postoperative pain scores/morphine requirements and c.118A>G genotype in patients undergoing laparoscopic procedures. The same study did, however, show an association between higher opioid doses and homozygote carriers in the chronic pain population. In addition, clinical studies involving the cancer population have also shown a tendency towards increased morphine requirements in individuals homozygous for the c.118A>G allele.

β-Arrestin
Genetic variation in intracellular signalling pathways may also influence the clinical response to opioids. β-Arrestin is a regulatory protein involved in the desensitization of opioid receptors after prolonged exposure to agonist drugs. Animal studies have shown potentiation and prolongation of the effects of opioids in mice lacking the β-arrestin gene. Clinical studies in cancer patients show an association between the β-arrestin 2 gene variant and a poor tolerance to morphine necessitating opioid switch.

Catechol-O-methyltransferase
COMT regulates the metabolism of catecholamines such as norepinephrine, epinephrine, and dopamine; thus, mutations in the COMT gene can effect the perception of pain. Reduced COMT activity leads to larger levels of catecholamine neurotransmitters and increased pain sensitivity in animal models of pain. A common human polymorphism in the COMT gene, p.V158M (Val158Met in alternative nomenclature), leads to a three–four-fold variation in COMT activity, with p.158MM homozygous individuals demonstrating the lowest COMT enzyme activity and subsequent reduced activation of the endogenous opioid system and compensatory up-regulation of opioid receptor expression.

Although low COMT activity is associated with increased pain sensitivity, clinical studies of COMT polymorphisms suggest that these individuals require smaller opioid doses, possibly due to up-regulation of opioid receptors. Among the cancer population carriers of the COMT, p.158VV and p.158VM genotype have been shown to require 63% and 23% higher doses of morphine compared with carriers of the p.158MM genotype. In the postoperative pain setting, Kim and colleagues failed to show a significant association between COMT polymorphisms and pain responses, although a low frequency of some polymorphisms and lack of statistical power may have contributed to this result.

Melanocortin-1 receptor
The melanocortin-1 receptor (MC1R) is thought to play a role in mediating kappa-opioid receptor sensitivity. Mogil and colleagues demonstrated that women with variant MC1R alleles demonstrated altered sensitivity to the kappa-opioid agonist pentazocine. Individuals with pale skin and red hair are more likely to carry inactivating variants of the MC1R gene and demonstrate reduced sensitivity to noxious stimuli and increased analgesic response to morphine-6-glucuronide.

Pharmacokinetics
Opioid absorption and distribution
Drug transporter proteins facilitate the passage of opioid drugs across biological membranes in organs such as the liver, kidneys, intestines, and at the blood–brain barrier. Systems involved in both the efflux and the uptake of drugs have the potential to influence the absorption, distribution, and elimination of opioids. Genetic polymorphism in these transporter systems may, therefore, account for some of the inter-patient variability in response to opioid drugs.

Many opioids are substrates for the P-glycoprotein (P-gp) efflux transporter, including morphine, fentanyl, methadone, and sufentanil. P-gp protein expression varies widely among individuals and the gene encoding P-gp is highly polymorphic. Two common SNPs at position 3435 and 2677 in the gene encoding P-gp have been investigated for their effects on opioid pharmacokinetics. Pharmacokinetic modelling of morphine in neurosurgical patients has shown that a 3435 homozygous mutant genotype was associated with significantly increased maximum cerebrospinal fluid concentrations of morphine. However, clinical studies have shown no association between the 3435 SNP and postoperative morphine
requirements.\textsuperscript{13} Investigations of combination SNP variants such as the 2677 and 3435 haplotype have found associations with opioid side-effects.\textsuperscript{13} In addition, when haplotypes with additional SNPs at other loci were investigated in methadone-dependent individuals, an association with higher methadone doses was seen.\textsuperscript{11}

Proteins implicated in the uptake of opioids, such as the SLC organic anion-transporting polypeptides, may play a role in determining the distribution of opioids in organs such as the liver and brain. Although genetic polymorphisms of genes expressing these proteins have been described, clinical studies investigating the consequences of this are lacking.\textsuperscript{40}

\textbf{Opioid metabolism}

Opioid metabolism by cytochrome P450 enzymes and enzymes that regulate glucuronidation to active metabolites influence the concentration of drug available at the effect site and therefore its clinical efficacy. Genes encoding enzymes responsible for these metabolic pathways exhibit considerable polymorphism resulting in wide variability in inter-individual responses to some opioids.

The clinical effects of the weaker opioids codeine, dihydrocodeine, tramadol, oxycodone, and hydrocodone rely upon the formation of more potent hydroxyl metabolites (such as morphine, dihydromorphine, and oxymorphone) by a metabolic pathway mediated via the cytochrome P450 enzyme CYP2D6.\textsuperscript{74} The gene encoding CYP2D6 exhibits significant polymorphism, with 100 variant alleles identified.\textsuperscript{74} Variant allele expression results in a number of clinical phenotypes: poor metabolizers express two non-functional alleles (such as 4*, 5*, and 6*), intermediate metabolizers express at least one reduced functional allele (such as 9*, 10*, and 41*), and extensive metabolizers express at least one functional allele.\textsuperscript{88} A fourth phenotype, the ultra-rapid metabolizer occurs when a gene duplication event results in the individual having multiple copies of the functional allele.\textsuperscript{47} \textsuperscript{88} Approximate frequencies of these phenotypes for the Caucasian population are: poor metabolizers 5–10\%, intermediate metabolizers 10–15\%, extensive metabolizers 65–80\%, and ultra-rapid metabolizers 5–10\%.\textsuperscript{88} The prevalence of variant alleles exhibits considerable inter-ethnic differences, for example, the frequency of CYP2D6 gene duplication associated with ultra-rapid metabolism ranges from 0.5\% in China to 29\% in Ethiopia.\textsuperscript{27}

Codeine is a prodrug whose action is determined by demethylation to morphine, a reaction catalysed by the enzyme CYP2D6. A number of \textit{in vivo} studies have demonstrated detectable differences in plasma morphine concentrations between extensive and poor metabolizers.\textsuperscript{7} \textsuperscript{8} \textsuperscript{87} Yue and colleagues\textsuperscript{87} found that more than five times less morphine metabolites were excreted in poor metabolizers compared with extensive metabolizers after codeine 25 mg. Ultra-rapid metabolizers given codeine 30 mg demonstrated 50\% larger concentrations of morphine in the plasma compared with their extensive metabolizer counterparts in a recent study by Kirchheiner and colleagues.\textsuperscript{35} Laboratory investigations in healthy volunteers have shown little or no analgesia from codeine in poor metabolizer compared with extensive metabolizer groups exposed to electrical and cold pain.\textsuperscript{15} \textsuperscript{60} However, clinical investigations of CYP2D6 genotype in the postoperative pain setting have shown conflicting results, and well-designed prospective studies are lacking. In a small study of 11 patients given codeine after hysterectomy, two patients had no analgesic effect from the codeine, one of whom was subsequently shown to be a poor metabolizer.\textsuperscript{58} Other studies have shown no difference in postoperative pain intensity between extensive and poor metabolizer groups in both adults and children.\textsuperscript{51} \textsuperscript{85} Lack of clinical difference between genotype groups may be explained by the effects of other codeine metabolites such as codeine-6-glucuronide whose metabolic pathway is independent of CYP2D6, and has been shown to demonstrate central nervous system effects.\textsuperscript{25} \textsuperscript{82} Although Kirchheiner and colleagues\textsuperscript{35} noticed more codeine-related sedative side-effects in ultra-rapid metabolizers, in studies investigating extensive and poor metabolizers, codeine side-effects do not seem to be related to the CYP2D6 genotype.\textsuperscript{18} In conclusion, 5–10\% of Caucasian patients (poor metabolizers) are unlikely to gain full benefit from codeine administration, but are just as likely to suffer codeine-related side-effects, although these findings have yet to be confirmed in postoperative pain studies.

Tramadol exerts analgesic effects via the opioid agonist metabolite M1 (O-demethyl tramadol) and via modulation of noradrenergic and serotonergic monoamine pathways. O-demethylation of tramadol to the opioid agonist M1 is mediated by CYP2D6, and laboratory studies have shown significantly lower plasma concentrations of M1 in poor metabolizer compared with extensive metabolizer genotypes and subsequently reduced analgesic effects in experimental pain.\textsuperscript{19} \textsuperscript{55} However, although the opioid effects are reduced in poor metabolizers of tramadol, the enantiomer responsible for modulation of the monoamine pathways accumulates. Therefore, the monoamine analgesic effect increases.\textsuperscript{16} The clinical consequences of these differences have been investigated in the postoperative pain population. In a prospective study, Stamer and colleagues\textsuperscript{76} 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 metabolizer group compared with the extensive metabolizer group.\textsuperscript{76} Subsequent studies have confirmed the high non-response rate and increased dose requirements in patients with a poor metabolizer genotype.\textsuperscript{77} \textsuperscript{83}

Oxycodone is O-demethylated via the CYP2D6 enzyme to the potent \(\mu\)-opioid agonist oxymorphone. However, this pathway only accounts for 11\% of the orally administered drug and the principal metabolic pathway in humans is
N-demethylation via the enzyme CYP3A. Nevertheless, in the experimental pain setting, analgesic response to oxycodone is inversely correlated with CYP2D6 phenotypes.

Similarly, methadone is metabolized by both CYP2D6 and CYP3A pathways. CYP2D6 genotype influences methadone steady-state plasma concentrations, and a reduction in the success rate of opioid substitution treatments may be related to poor metabolizer status. The CYP3A metabolic pathways play an important role in the metabolism of other opioids commonly used by anaesthetists including alfentanil and fentanyl. However, although genetic polymorphism occurs in the enzyme CYP3A4, unlike CYP2D6 this has not been correlated with particular clinical phenotypes.

Phase II metabolism of some opioids is catalysed by uridine diphosphate glucuronosyl transferase (UGT). UGT is responsible for the glucuronidation of morphine to morphine-6-glucuronide and morphine-3-glucuronide metabolites and has a role in codeine metabolism. Although genetic variations in the UGT enzyme have been described, the clinical consequences have not been fully elucidated.

**Pharmacogenomic studies of other anaesthetic drugs**

There are many drugs prescribed and administered by anaesthetists, but it is not possible to cover the evidence for pharmacogenomic variability in all of them in this article. Instead we will focus on the relatively limited literature concerning the pharmacogenomics of inhalation and i.v. anaesthetics. We will discuss the reasons for this paucity of studies below, but it is worth highlighting that variability of response to general anaesthetics is likely to play an important role in the incidence of awareness under anaesthesia.

**Genetic determinants of anaesthetic response**

Evidence from animal experiments suggests that at least some of the variability in response to anaesthetic agents results from genetic factors. For example, in-bred mouse strains differ in their MAC values for potent inhalation anaesthetics and in the concentrations of barbiturate, ketamine, or nitrous oxide to inhibit the righting reflex. Cascio and colleagues have even identified a locus on mouse chromosome 7 associated with MAC. Human studies, however, are limited; this may reflect the difficulty in defining phenotypes for anaesthetic response that can be used reproducibly in studies involving relatively large numbers of subjects or patients. The Wellcome Trust case–control consortium, for example, attributed the lack of association in their genome-wide association study of hypertension to difficulties with defining the phenotype. Another factor, especially in the case of potent inhalation anaesthetics, is the difficulty in identifying genes that are potentially associated with anaesthetic response. Iohom and colleagues, for example, identified the genes encoding the epsilon subunit of the GABA receptor (GABRE) and the CYP2B6 gene as candidates for the pharmacodynamic and pharmacokinetic variability of propofol, respectively. They used clinical and electroencephalographic assessments of onset of anaesthesia, clinical recovery time, and drug clearance as response variables. No associations were found but, in common with the approach of many pharmacogenomic studies, these authors may have diminished the power of their analyses by converting their outcome data to a categorical level rather than analysing it as a quantitative trait. There were, however, further problems with this study, such as the age range of the patients (18–74 yr) and the lack of conformity with Hardy–Weinberg equilibrium of some of the genotyping data, which may well irredeemably confound any prospect of a meaningful re-analysis.

A seemingly speculative gene association study did, however, find an association between variants in the MC1R gene (i.e. red-haired individuals) and dose requirement of desflurane to prevent response to a noxious stimulus. The authors of this study could not provide a satisfactory explanation for the 19% difference in desflurane MAC between red- and dark-haired subjects, but they may have underestimated the bias inherent with a non-blinded observer. There is also a genetic confounder that they did not consider and this is known as population stratification. Here, a characteristic may be more common in a subpopulation and this characteristic will be associated with any genetic marker that is more prevalent in this subpopulation than other sub-populations, even if the genetic marker plays no role in determining the characteristic. Furthermore, this association is not dependent on the proximity of the genetic marker and the gene determining the characteristic. The red-haired subjects in the study of Liem and colleagues may, therefore, emanate from a population that is genetically distinct from the dark-haired subjects. Interestingly, Ezri and colleagues have demonstrated an association between ethnicity and sevoflurane requirements.

**Pharmacogenomic variability and adverse responses to anaesthetics**

**Malignant hyperthermia as a complex genetic trait**

Although MH was historically described as apparently autosomal dominant, there is consistent evidence that MH is a disorder with a complex molecular genetic basis; this has delayed the implementation of widely applicable DNA-based screening. Almost 200 genetic variants in RYR1, the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel, have been reported in MH. Our analyses of independent MH families in the UK have so far detected a total of 66 RYR1 mutations, with ~50% private to individual families. This pattern is repeated across other populations worldwide.
In addition to multiple RYR1 variants, five other chromosomal loci show possible linkage to MH. Although mutations have been found in only one gene (CACN1AS, encoding the principal subunit of the voltage sensor of the skeletal muscle T-tubular membrane) located in these regions, and then in only a handful of families, we have sequenced all 106 exons of RYR1 in 192 MH-susceptible individuals and found no mutation in 50 (~25%) of them. This suggests that variants in genes other than RYR1 are responsible for MH susceptibility in an appreciable minority of cases.

To add further complexity, data collected from laboratories across Europe demonstrated that ~5% of individuals with familial RYR1 variants are not susceptible to MH. Similarly, ~2.5% of families carrying an RYR1 variant contain one or more MH-susceptible members without the familial RYR1 variant. Genotyping and phenotyping errors have been excluded as a cause of these discordant results, which can be explained by the action of other genes either contributing to susceptibility or modifying the effects of RYR1 variants. Furthermore, using a type of association analysis known as transmission disequilibrium testing, we have demonstrated in UK families and validated in European pedigrees that MH status may be conferred by multiple interacting gene products, including those where a RYR1 variant is operating. This evidence is consistent with the inheritance of MH being determined by a threshold model with RYR1 being a major but not obligatory factor. Contributing non-RYR1 variants could possibly be relatively common with a relatively minor contribution to the phenotype or relatively uncommon with a greater contribution to the phenotype.

Nitrous oxide toxicity

Prolonged exposure to nitrous oxide is known to be associated with megaloblastic anaemia, agranulocytosis, and neuropathy secondary to acute demyelination. This results from inactivation of vitamin B12 by nitrous oxide. Vitamin B12 is an essential cofactor of methionine synthase, which is a crucial enzyme in the folate, methylation, and trans-sulphuration cycles. Recent cases have drawn attention to rare catastrophic acute demyelination after relatively short durations of exposure to nitrous oxide in children with variants in the gene encoding 5,10-methylene tetrahydrofolate reductase (MTHFR). Nitrous oxide should not be administered to patients known to have MTHFR deficiency or those with a family history of the condition. Furthermore, Nagele and colleagues have demonstrated raised plasma homocysteine concentrations after >2 h nitrous oxide exposure in patients homozygous for either of two common variants in the MTHFR gene. The clinical relevance of these observations is unclear, but if transiently increased plasma homocysteine concentrations do prove to be associated with detrimental clinical outcomes, fully informed risk–benefit decisions concerning the use of nitrous oxide in individual patients will require preoperative genotyping of MTHFR gene variants.

Conclusion

Candidate gene association studies have identified variants in the OPM1 and CYP2D6 genes as the most likely factors identified so far to possibly influence opioid responses in the perioperative setting. The lack of definitive and confirmatory data probably reflects the weakness in the candidate gene approach when many other genetic factors may be operating. The practical difficulties posed by the design requirements for a genome-wide association study using a clinical opioid response phenotype are considerable. Similar study design problems apply to the investigation of the pharmacogenomics of i.v. and inhalation anaesthetics by genome-wide association. It will be interesting to see if the anaesthesia research community can overcome these difficulties and then persuade funding bodies to provide the large grants required to support them.

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