Omics and anaesthesia: pharmacogenomics, proteomics and metabolomics

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Abstract

The inter-individual response to medications and the presence of genetic polymorphisms which impact the safe conduct of anaesthesia and analgesia are of paramount importance to the anaesthetist, intensivist and pain physician. The frequency of these phenomena is not reflected in the attention afforded them in undergraduate or postgraduate curricula. In order to appreciate how these issues may affect our clinical practice, it is crucial to have a working understanding of the concepts that underpin the relevant fields within the collection of disciplines we term the 'omics'.

Keywords Cytochrome; metabolomics; neurotransmitter; Omics; pharmacogenetics; pharmacogenomics; proteomics; receptor; single nucleotide polymorphism; transporter

Royal College of Anaesthetists CPD Matrix: 1A01, 1A02

Learning objectives

After reading this article, you should be able to:

- define pharmacogenomics, proteomics and metabolomics
- demonstrate the implications of polymorphisms on the variable response to drugs
- discuss common polymorphisms and how they impact drug choice on a clinical level
- propose the future roles of research in these fields and how this will inform our practice

Background

Personalized medicine had a sudden explosion of interest at the turn of the millennium with a move from a 'one size fits all' to a more individualized practice of medicine sought with increasing vigour. That the Human Genome Project (completed in 2003) straddled this upsurge in attention is no coincidence.[1] The ability to map a person's genetic make-up had great potential for learning more about how this might affect the variable response to medications we had observed in the preceding years.

By the mid-1990s, fields such as genomics, proteomics and metabolomics, having little previous attention in the academic literature, began to flourish. Since then these disciplines have been applied to areas as diverse as the search for new drug targets in disease and the isolation of individual female-attracting pheromones in male mouse urine.[2]

In short, the past two decades have seen a steep rise in the role of these new areas. While this is yet to filter down to true, personalized medicine on an individual level, it is greatly informing such thinking in research and development. This can only be encouraging that the future holds advances capable of changing the way we approach the individual for the better.

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Pharmacogenomics

In true *omic* nomenclature, genomics is the study of the whole genome, where genetics pertains to individual genes. Similarly, pharmacogenomics is the study of the whole genome's effect on pharmacological response. Pharmacogenetics looks at individual genes. Often these terms are used interchangeably.

An individual drug response may differ in a number of ways. Largely, these can be categorized into pharmacokinetic (the body's effects on the drug) and pharmacodynamic (the drug's effects on the body) variables. Alteration in genetic material can bring about vast changes in these variables, from complete inability to utilize the drug to hypersensitivity and toxicity. To understand how these differences come about, we must revisit some basic genetic principles (Table 1).

Genetic material is held in chromosomes made up of deoxyribonucleic acid (DNA). The familiar double helical structure comprises four nucleotide bases: two purines (adenine, guanine) and two pyrimidines (thymine, cytosine); they are commonly expressed as their letter form — A, G, T, C. These bases pair up across the helix in a consistent manner: A with T, G with C. Their role is to code for the production of amino acids — the building blocks of proteins. They do this by forming genetic sequences. A collection of three bases is called a codon and these three letter genetic 'words' code for a particular amino acid. There are 20 standard amino acids, but many more potential combinations of bases, meaning that each amino acid may be coded for by a number of different codons. Any changes to the sequence of bases, therefore, may or may not alter the amino acid produced (non-synonymous versus synonymous).

A gene is made up of a variable number of codons, with some composed of a few hundred bases while others contain millions. Only around 1% of DNA actually codes for a protein. The remainder is termed non-coding DNA. This was historically assumed to be entirely genetically inert, but more recently has been shown to have an important role in controlling gene activity. The ends of chromosomes (telomeres) are non-coding and have a protective role in preventing chromosomal degradation during copying. Coding parts of DNA are called exons and so the coding portion of the genome is termed the exome.

The most frequent changes seen in DNA sequencing are termed single nucleotide polymorphisms (SNPs): a change in one sole base by insertion, deletion or substitution. Adding or

A recap of basic genetic terms and descriptions

Term	Description
Chromosome	Thread-like structure made up of a DNA molecule, often pictured following replication when two sister chromatids join at a centromere, producing an X-shaped formation Usually 23 pairs in humans, the last of which confers sex dependent on X or Y inheritance
Gene	A DNA (or RNA) sequence that codes for a functional molecule
Locus	The specific location of a DNA sequence or gene on a chromosome
Allele	A variant of the sequence of DNA at a given locus
Genome	The complete set of coding and non-coding DNA: species specific — e.g. *Homo sapiens* vs *Pan troglodytes*
Genotype	An organism's complete set of hereditary information: individual specific — e.g. Dave vs Jim — Identical twins have the same genotype
Phenotype	The physical characteristics as manifestations of an individual's genotype — Identical twins have different (often similar) phenotypes
Haplotype	A group of genes/alleles that tend to be inherited together — from the same chromosome and same parent — *Portmanteau* of haploid and genotype
Polymorphism	A gene with more than one allele
Single nucleotide polymorphism	An allele produced due to variation in one base unit

Table 1

subtracting a nucleotide will not only change the meaning of the 'word'/codon concerned, but also shift the remaining sequence along by one base — this completely alters the 'sentence' as well as the 'word' (Figure 1).

Pharmacogenetics and pharmacogenomics

As mentioned above, there is a subtle difference between these two fields in that pharmacogenomics studies the whole genome whereas pharmacogenetics analyses individual genes. For example, pharmacogenomics utilizes genome-wide association studies (GWAS) to identify phenotypic differences to account for the inter-individual variability seen in drug response. In contrast, pharmacogenetics focuses largely on the differences in drug metabolism brought about by SNPs and other genetic variables. This is well illustrated by examples from pain management.

Drug metabolism variability

Drug metabolism comprises two main phases. Phase I is termed the modification phase, where initial drug metabolism occurs via oxidation (most commonly), reduction or hydrolysis reactions.

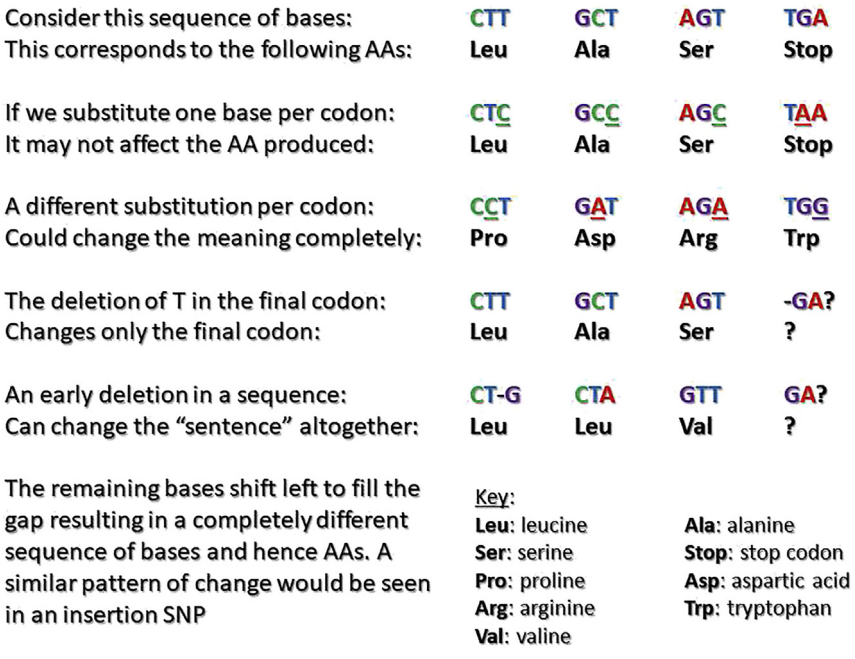

Figure 1 Examples of substitution and deletion SNPs and effect on amino acid (AA) coding

Phase II involves conjugation with certain charged species via enzymatic reactions (e.g. through glucuronidation, methylation, sulphation or acetylation). The addition of molecular weight during phase II often renders the molecules inactive, whereas the production of active metabolites is more common in phase I reactions. Some metabolites (e.g. glutathione conjugates) require a further processing step termed Phase III.

The cytochrome P450 (CYP450) family of hepatic enzymes comprises 59 proteins which perform up to 80% of drug metabolism. CYP450 2D6 (CYP2D6) is responsible for the metabolism of certain substances including codeine and amitriptyline. It has been known for many years that there is a wide variability in response to codeine administration. Codeine requires CYP2D6 to convert it to the active metabolite, morphine. Alterations in the function of CYP2D6 caused by SNPs therefore have implications for the effectiveness of codeine in the individual. While the majority of Caucasians are extensive metabolizers (being homozygous with two functional alleles), around 10% are poor metabolizers, which results in low conversion to morphine and poor efficacy (no functional alleles). A further 10% are intermediate metabolizers (heterozygous with a functional and non-functional pair of alleles) and 10% ultra-rapid metabolizers with high conversion to morphine and potential toxicity (possess 3 or more functional alleles). Without prior knowledge or genetic testing it is difficult to predict response, causing serious limitations in the drug's utility.[3]

Meanwhile, the tricyclic antidepressant amitriptyline is variably metabolized by CYP2D6 to the less active 10-hydroxy metabolite, and by CYP2C19 to the active metabolite nortriptyline. Amitriptyline (and nortriptyline) are frequently prescribed in low doses for the treatment of neuropathic pain. In those patients with poor CYP2D6 metabolism, the parent compound will have a longer half-life and the potential to accumulate. Moreover, the metabolism will swing more towards the CYP2C19 arm, producing nortriptyline as an active metabolite. It can be seen that only very low doses are required to exhibit the desired effect, while side effects can be very problematic at an early stage of treatment. With ultra-rapid metabolism, very high doses may be required to maintain therapeutic plasma levels. Again, the individual response is unpredictable, leading to a requirement for surveillance around the initiation of therapy.[4]

Interestingly, CYP2C19 is also susceptible to poor metabolizer variants — this has further implications on its role in the metabolism of omeprazole and clopidogrel. This is particularly pertinent if these two medications are used concomitantly. Clopidogrel is a prodrug, requiring conversion to the active compound by CYP2C19 (and other pathways). In CYP2C19 poor metabolizers, conversion to the active metabolite is reduced and the antiplatelet effect is diminished. While platelet mapping can be performed as a bedside test to investigate the efficacy of clopidogrel and other antiplatelet agents, it is not usual and is of questionable utility for informing drug dosing. Up to 14% of the population are thought to be CYP2C19 poor metabolizers and are, therefore, at risk of cardiovascular events intended to be prevented by the administration of clopidogrel. The situation can be further compromised by the addition of enzyme inhibitors. Omeprazole inhibits CYP2C19, resulting in yet lower conversion of clopidogrel to its active metabolite. It is therefore recommended that the two medications should be avoided in combination.[5]

Drug transport variability

Transporter proteins facilitate transfer of substances across biomembranes (e.g. the blood–brain barrier) and can be crucial to a drug's ability to reach the effect site. A useful example is the adenosine-triphosphate binding cassette (ABC) superfamily of transporters. This group of proteins is subject to variability in the same way as CYP — via SNPs affecting the coding genes. Functional variability in ABCB1 can result in altered antidepressant and steroid transport, as well as a clinically relevant increase in ondansetron bioavailability leading to improved response in postoperative nausea and vomiting.[6]

Receptors, neurotransmitters and immune components

Genetics may impact pharmacology in a number of other ways, including the function of target receptors, components of the immune system and the metabolism of neurotransmitters (which may form a part of a drug's mechanism of action). Both receptor and neurotransmitter variability are implicated in the variable response seen to opioid administration.

The *OPRM1* gene codes for the μ-1 opioid receptor, the site of action of morphine and other opioid medications. Abnormalities in the function of this gene can result in altered response to opioids and, hence, differences in pain score. The classic example is the A118G SNP, an A to G substitution resulting in increased postoperative opioid requirements as well as having an impact on postoperative nausea and vomiting.[7]

Meanwhile, catechol-O-methyl transferase (COMT) is an enzyme responsible for the inactivation of catechol neurotransmitters by the addition of a methyl group. SNPs resulting in amino acid substitution from methionine to valine have been shown to increase morphine requirement. Interestingly, this enzyme has also been associated with an increase in pain experience more generally and with the incidence of fibromyalgia.[7]

The human leucocyte antigen (HLA) group is the human form of the major histocompatibility complex (MHC) family of genes, coding for cell-surface proteins which present intracellular peptides to the immune system. On presentation, there is recognition of material as host (and therefore safe), or foreign, resulting in an immune reaction (e.g. triggering apoptosis). Alleles of the *HLA* genes number in the hundreds, providing a wide variety of inter-individual immune response. Some alleles, however, are implicated in unwanted immune mediated reactions to medication. An example is the increased risk of Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) to administration of carbamazepine in those with the *HLA-B*1502* allele. This allele has higher incidence in those of Han-Chinese, Thai and Malaysian origin.[8] SJS/TEN manifest as initial flu-like symptoms followed by the eruption of a blistering skin rash similar to a severe scalding injury. The condition characteristically also affects the mucous membranes, eyes, genitalia and the palms and soles. It has been suggested that genetic testing should be undertaken in those at risk through their ethnicity prior to establishing on this medication. Aside from being a commonly prescribed anti-epileptic, carbamazepine is used with great effect in trigeminal neuralgia and less so in other facial pain disorders and neuropathy with lancinating features.[9]

Pharmacogenomics specific to anaesthesia

Genetic variation can play a minor, sub-clinical role in response to anaesthetic agents, but may also lead to potentially fatal complications.

Malignant hyperpyrexia (MH): is a hypermetabolic condition characterized by temperature rise, muscle rigidity and hypercapnoea. It is most frequently due to a polymorphism of the ryanidine receptor gene *RYR1* coding for the ryanodine receptor, a calcium releasing channel mainly found on the sarcoplasmic reticulum (SR) of skeletal muscle. This calcium flow promotes coordinated muscle contraction. Polymorphisms of *RYR1* (approximately 70% of MH cases – MHS1) and those affecting CACNA1S (approximately 1% of cases – MHS5), which helps to activate RYR1, can result in the channel opening more easily or being slower to close. This occurs on exposure to certain agents: suxamethonium and volatile anaesthetics (e.g. sevoflurane, isoflurane). MH is inherited in an autosomal dominant pattern, with *RYR1* located in chromosome 19. Those with a family history should undergo genetic testing in a specialist centre prior to elective surgery. In the emergency setting a vapour-free anaesthetic machine and avoidance of suxamethonium should be utilized.[7,9]

Suxamethonium apnoea: is characterized by prolonged neuromuscular blockade following administration of suxamethonium (succinylcholine) due to dysfunction of its metabolism by plasma cholinesterase. The degree of prolongation is variable depending on the allele present on chromosome 3, inherited autosomally. Several alleles exist: normal is termed $E1^u$ (usual), $E1^a$ is abnormal, $E1^f$ fluoride-type and $E1^s$ silent-type. Homozygotes of $E1^u$ ($E1^uE1^u$) have a normal plasma cholinesterase activity and a short duration of suxamethonium action (2–6 minutes). Heterozygotes of the abnormal allele ($E1^u$ $E1^a$) display approximately 30-minute duration of action, whereas $E1^a$ homozygotes can have greater than 2-hour duration. Fluoride and silent types are rarer and can exhibit even more prolonged recovery times. Clearly, this can have significant implications on the individual and on the running of a theatre list, with an unexpected apnoea causing delays in recovery and potentially the need for admission to intensive care for ongoing invasive ventilation and sedation to prevent awareness. It should be noted that a similar pattern can be seen with mivacurium use due to its metabolism by plasma cholinesterase.[7,10]

Propofol dosing variability: aside from the extreme examples above, there are incidences of variability in response to drugs used in anaesthesia which may go untested or unnoticed. Propofol (2,6-diisopropylphenol) is a very commonly used intravenous induction agent. It potentiates GABA resulting in rapid onset general anaesthesia, with sedation at lower doses and is frequently utilized in intensive care and in target-controlled infusion as a component of total intravenous anaesthesia (TIVA). What may limit its use, particularly as a rapid sequence induction agent, is the variable dose required to achieve anaesthesia. Part of this variability can be traced to genetics. Uridine 5′-diphospho-glucuronosyltransferases (UGT) is another group of conjugation enzymes responsible for the glucuronidation of certain endogenous substances (e.g. thyroid hormones) and many drugs, including propofol. UGT1A9 is the enzyme specific to propofol conjugation and polymorphisms in the gene coding for this protein, for example a T to G substitution SNP at locus 1887, can lead to an increase in required induction dose and prolonged time to effect. UGT is also partly responsible for paracetamol metabolism whereas UGT2B7 metabolizes naloxone. The cat's lack of the enzyme results in toxicity to many drugs which are safely administered in humans.[7]

Local anaesthetic resistance: voltage-gated sodium channels (Na_v family) play a vital role as one of many propagators of a nociceptive action potential. Disorders in this family, caused by genetic polymorphisms, can result in alterations in pain perception. A good example is in the *SCN9A* gene, coding for $Na_v1.7$, which cause erythromelalgia, characterized by spontaneous paroxysms of intense pain. As is well documented, local anaesthetics function by their blockade of sodium channels, impeding propogation of the action potential. There are several reports of failure of local anaesthetic agents in those with polymorphisms in the Na_v family, including erythromelalgia. A publication which received much press attention reports the possibility of local anaesthetic resistance in those with a $Na_v1.5$ dysfunction due to *SCN5A* polymorphism. This particular paper was the result of whole exome sequencing of family members, some of whom exhibited resistance to local anaesthesia.[11]

Proteomics

The Human Genome Project saw the publication of a sequence containing some 19,599 protein coding genes. The number of proteins and protein fragments produced following transcription and translation, however, far outnumbers this – perhaps over 1 million. For this reason, proteomics – the analysis of the entire protein complement of a cell/tissue/organism – can be considered more complex. This is emphasized particularly when we realize that the proteome, unlike the genome, is variable over time and according to the cell or tissue type concerned.[1,9]

Transcription is the process by which the DNA sequence is copied into a messenger ribonucleic acid (mRNA) structure by RNA polymerase. This occurs in the cell nucleus and the resultant mRNA can then travel into the cytoplasm to undergo translation. Ribosomes in the cytoplasm or on endoplasmic reticulum decode the mRNA sequence to produce a polypeptide chain, which results in the formation of a protein. Chemical modification of the post-translational polypeptide by phosphorylation and ubiquitination (by the small protein ubiquitin) is essential to the correct functioning of the protein produced. It can be seen, then, that multiple changes are possible which can result in a large number of protein-based structures, all of which are studied in proteomics. The relationship between genomics and proteomics, as well as the other fields covered here can be seen in Figure 2.

The role of proteomics in medicine is to search for those proteins which are associated with disease by way of their altered expression or post-translational modification, and to discover new protein targets for diagnostic or pharmacological purposes.

When applied to anaesthesia, the proteomic field is focused on two main areas in the literature. Firstly, on the discovery of drug targets, for example, specific effect sites for volatile agents and novel pathways/potential receptors for analgesics. Secondly,

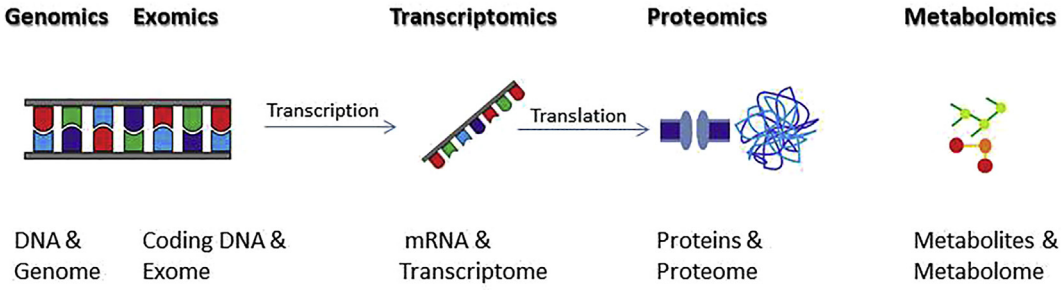

Figure 2 Hierarchy of the omics (Adapted from Lambert, 2016).

on the ways in which anaesthesia as a vehicle to achieving a set goal can, in fact, be complicating the matter. For example, much laboratory research in proteomics and other fields is conducted by collecting samples from animal subjects. This may involve anaesthesia to allow safe and humane sampling of body fluids or tissues, or euthanasia of the subject, often by use of the same anaesthetic agents. The complicating factor is that the anaesthetic agents themselves may be causing some of the proteomic differences that would otherwise be attributed to the disease state or pharmaceutical being researched. Similarly, a study of the response to anaesthesia in human subjects undergoing electroconvulsive therapy (ECT) for psychiatric disorders found that proteomic variables previously ascribed to ECT were, in fact, brought about by exposure to the anaesthetic agents used.[12]

More recently, research has focused on the differential effects on the proteome of intravenous versus inhalational agents. Publications have shown that both propofol and volatile anaesthetics produce proteomic changes which are discoverable in the laboratory. Interestingly, these changes are inconsistent between the agents. It appears that propofol is less disruptive to the proteins it alters than is sevoflurane, isoflurane or dexmedetomidine. Proteins associated with apoptosis and neurodegenerative disease were downregulated by propofol and those impacting on cell signalling and cytoskeletal growth were less affected than with the volatiles. What this equates to in clinical practice is not yet certain, though these are clear areas for future research opportunities.[13,14]

Metabolomics

Metabolomics is defined as the study of the entire set of metabolites within in a cell/tissue/organelle following a specific cellular process. These metabolites can include glucose, lactate, glutamate and other amino acids. Traditionally, metabolite concentrations would be measured using gas or liquid chromatography and mass spectrometry. This involves actual tissue sampling and can only be achieved following the event in question and may involve the administration of anaesthesia or even euthanasia to facilitate sampling. As described above, both of these methods can produce confounding effects on data. By using proton magnetic resonance spectroscopy (^{1}HMRS), metabolic substances can be traced real-time during particular activity or while administering particular medication without the need for tissue sampling. In this way, the metabolic response to certain agents can be sought which may be useful in determining the location of effect sites and the likelihood of sequelae.

For example, in 2012 Jacob et al. applied ^{1}HMRS to trace metabolites in the brains of children being anaesthetized with sevoflurane or propofol to facilitate MRI scanning. They found that the cerebral metabolomic profile of the children was significantly different dependent on the agent used. Propofol was associated with lower signal from glucose and lactate than was sevoflurane. Regression analysis was undertaken to determine if this could be implicated in the differences in emergence delirium observed between the agents. The result was that emergence delirium was less commonly seen with propofol than sevoflurane, and that this appeared to be related to the lower concentration of lactate in the parietal cortex. This and similar evidence could have the potential to inform anaesthetic choices in the future.[15]

It can be seen that proteomics and metabolomics are complementary. Metabolites studied are not restricted to small endogenous compounds but can include the metabolic products of drugs, many of which are produced by the action of enzymes.

Summary

While a complete shift towards personalized medicine has its branches some way in the future, it can be seen that at least some of its roots lie here in current omics research and development. Through familiarity with the principles covered in this article, we can begin to think in a more individualized manner about our patients' care in the anaesthetic, intensive care and pain management environments. As pharmacogenomic, proteomic and metabolomic studies continue, we may well find answers to age old questions about anaesthetic and analgesic action, while discovering new targets to question going forward. ◆

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