Editorial

Pharmacogenomics and anaesthesia: explaining the variability in response to opiates

Clinical experience tells us that there is great heterogeneity in the way patients recover from uncomplicated anaesthesia, as well as in their requirements for postoperative analgesia. The basis of such interindividual variations has, until recently, been poorly understood by clinicians. However, the recent quantum leaps in biotechnology that have led to improved understanding of the human genome have also permitted some insight into those subtle elements underlying variable drug effects, some of which are of mere academic interest, but some of which are an important cause of patient morbidity. Recently, considerable effort has been made to try and ascribe a function to each of the 30 000 or so genes that make up the human genome. Having identified a particular function for the gene, it may then be possible to identify subtle abnormalities in the DNA sequence. The simplest type of variation or polymorphism derives from a single base mutation in DNA that substitutes one nucleotide for another. This is called a single nucleotide polymorphism or an SNP (colloquially termed a snip). The pivotal importance of SNPs was recognized soon after their discovery, and it has become a key objective to map all of these variants across the entire human genome [1]. The focussing of vast resources on this task has been labelled the Snip-revolution and, to date, several million SNPs have been described [2].

The effect of any particular SNP i.e. the resulting phenotype, will, however, depend on the impact of the resulting substitution of the encrypted amino acid on the respective protein. This effect will vary depending on both amino acid substituted and its position. On one hand, there may be no apparent effect; on the other hand, the resulting protein may be fatally flawed with complete loss of function. Having detected an SNP, it is a straightforward step, using gene-association studies to link the abnormal gene with either a disease process or with an abnormal response to a medication. In the case of proteins that have been extensively characterized, such as the P450 enzymes, exhaustive databases have been complied, which include descriptions of a large number of SNPs and their respective phenotypes [3].

The variable clinical response to all drugs, including opiates, may be approached by considering the effect of genetic variation on both receptors (pharmacodynamics) and on factors that determine drug concentration (pharmacokinetics). For example, mutations in the gene that encodes the μ-opioid receptor OPRM1 are primary candidates for genetic influences on opioid effects. A much quoted polymorphism in this gene consists of an SNP at position 118 in the DNA chain of base-pairs [4]. The variant receptor protein derived from this gene exhibits altered binding with β-endorphin compared with the more common i.e. wild-type allele. A number of research groups have investigated this polymorphism and have found not only decreased opioid activity in carriers of the SNP resulting in altered analgesic requirements, but also differences in the incidence of side-effects, including less-associated nausea and vomiting. This has been found to be related to one of the key active metabolites of morphine, morphine-6-glucuronide (M-6-G) [5]. Similar findings including less-effective analgesia and greater dose requirements have been confirmed for other opioids such as alfentanil [6]. Other SNPs of the μ-opioid receptor have been found to be associated with a greater tendency towards opiate addiction [7]. Other researchers focussing on other opioid receptors have found various behavioural disturbances including heroin addiction and anorexia nervosa, which were associated with mutations in the delta opioid receptor (OPRD1) [8,9].

Opiate-derived analgesia is determined by the level of phosphorylation of the μ-opioid receptor. This reaction is catalysed by G protein-coupled receptor kinases (GRK) and the subsequent interaction with the ubiquitously expressed regulatory

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protein β-arrestin. This protein, which is encoded by the gene *arr2*, plays a key role in the desensitization of receptors following prolonged exposure to its respective agonist including the development of tolerance following prolonged use of opiates [10]. The mechanisms underlying the development of opiate tolerance are, however, complex, with a number of co-regulatory factors such as the neurotransmitter glutamate and *N*-methyl-D-aspartate receptors playing a significant role [11]. Mice bred without the arrestin gene have enhanced morphine-induced analgesia [12]. Studies carried out in cancer patients receiving long-term morphine therapy found correlations between the efficacy of treatment and the incidence of mutations in the *arr2* gene [13]. Responses to pain may also be regulated by interactions between different brain areas and various neurochemical systems. For example, catecholamines play a role in modulating the response to sustained pain. Mutations in the catechol-*O*-methyltransferase (*COMT*) gene, which regulates the metabolism of catecholamines and is a regulator of adrenergic and dopaminergic pathways, significantly alter the sensory and affective responses to pain [14]. Recently, several snips in this gene have been found to be associated with alterations in the perception of pain from various causes and with the risk of developing a chronic pain syndrome [15].

Pharmacokinetics deals with altered drug availability and may be considered in terms of both absorption across membranes such as the gut, as well as drug metabolism to inactive, or in the case of prodrugs to active metabolites. One such area that has recently attracted attention is the study of those transmembrane proteins whose function is the energy-dependent export of drugs from cells particularly of the intestine and blood-brain barrier and whose role is pivotal in the development of multidrug resistance, particularly in epilepsy and cancer chemotherapy. P-glycoprotein (PGP) is one of the main efflux transporters. It is encoded by the multidrug resistant gene (*MDR-1*) and is of significance to anaesthetists due to the fact that some opiate analgesics including morphine, methadone, sufentanil and fentanyl are among its substrates [16]. If morphine is administered to mice, which are selectively bred to be deficient in PGP (i.e. gene knockout mice), they have an increased response to the drug [17]. In human beings, the central nervous system concentrations of drugs such as antiepileptics and antiretroviral drugs can be increased by administering a PGP inhibitor [18,19]. Similarly, the plasma concentration of orally administered morphine can be increased by simultaneous administration of PGP inhibitors. Consequently, the genetic variants of PGP have been the focus of

attention for researchers interested in altered opioid effects. One group in particular has investigated the *MDR-1* gene for polymorphisms and has found that one variant (C3435T) is indeed associated with decreased PGP activity in the gut as reflected by higher than expected plasma digoxin levels [20]. The discovery and characterization of such *MDR-1* variants is, therefore, potentially an important step in the identification and evaluation of individuals who may have an abnormal response to medications and, in particular, may help explain the spectrum of responses to opiates.

Drug metabolism is an important area, which may be the basis for abnormalities in drug action. It has been known for many years that around 5–10% of Caucasians have a defect in their ability to metabolize a number of commonly used drugs including the analgesics tramadol and codeine. The defect is due to an abnormality in one of the cytochrome P450 enzymes, namely CYP2D6 (formerly known as debrisoquine hydroxylase). It is now recognized that the enzyme defect stems from a mutation leading to faulty expression of the enzyme, and that so-called poor metabolizers, as opposed to extensive-metabolizers, either have complete deletion of the *CYP2D6* gene or, more commonly, have replacement of a single nucleotide leading to aberrant gene splicing [21]. There are a number of possible clinical sequelae for patients who are poor metabolizers of codeine. It is known that codeine is a prodrug, and its action is determined by the CYP2D6-dependent formation of morphine. In affected individuals, the ingestion of codeine results in undetectable or barely detectable amounts of morphine with little or no analgesic effect. In contrast, there is a genetic variation that occurs in some Caucasian, Middle Eastern and African populations, which have been shown to produce an 'ultrarapid metabolizing' phenotype. This phenotype results in abnormally high levels of morphine being produced from codeine. Such patients rapidly present with morphine-associated side-effects when standard doses of codeine are given [22]. Similarly, because tramadol is also metabolized by CYP2D6, its effects will be determined by the underlying genotype of the recipient. Other genes that exhibit genetic polymorphism and that are involved in the metabolism of opioids include the CYP enzyme 3A4 [23] and those that encrypt the glucuronosyl transferase group (UGT) of enzymes and, in particular, the sub-type UGT2B7, mutations of which are associated with abnormal patterns of morphine glucuronidation [24].

Increasingly pharmacogenomic differences between individuals are being characterized. Recently, amid great controversy, the anti-heart-failure drug Bidil,

directed at Afro-Americans, was introduced in the US and became the first medication directed at one particular racial group [25]. Recent technological advances, such as polymerase chain reaction and DNA microarray gene-chips, are providing clinicians with tests to rapidly assess altered patterns of gene expression that are increasingly being applied in various clinical situations [26]. For example, genotyping is now used routinely in some institutions prior to the use of potentially harmful medications, particularly in leukaemia [27]. In December 2004, the biotech company Roche Genomics introduced a commercially available microarray, *the Amplichip CYP450*, which allows clinicians for the first time to test patients for a wide spectrum of genetic variation in the genes controlling drug metabolizing enzymes including the CYP enzyme 2D6 [28]. Individuals who possess alleles other than the consensus, or reference allele, will now benefit from a personalized choice of medication with individual, or tailored dosage regimens allowing the prevention of untoward effects resulting from defective metabolism of the various substrates of this enzyme. Although, at present there are only a limited number of genes that can be easily assessed using commercially available tools such as microarrays, it is inevitable that such technology will continue to expand providing clinicians with the ability to examine all those genes relevant to drug action. Until recently, the unpredictable responses between individuals, racial groups and indeed between the sexes [29] have been poorly understood. Soon, in addition to *CYP2D6*, a spectrum of genes such as *CYP3A4, arr2, OPRM-1, MDR-1* and *COMT* may be examined allowing the dosage of opiates to be precisely tailored to the specific needs of individual patients.

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