European Journal of Anaesthesiology 2004; **21:** 505–508

ISSN 0265-0215

Editorial

Microarrays: new pharmacogenomic tools for the Twenty-first Century

The recent quantum leaps in genome-based knowledge have led to a number of significant changes in the direction of medical research and in particular the techniques involved in drug development. The successful mapping of the human genome [1] has facilitated the first steps in understanding the essence of interindividual as well as interspecies differences. For example, the genome sequences of human beings and chimpanzee are 98.8% identical [2] and 75% of the dog's genome is shared with that of human beings [3]. Even lower life forms such as the tiny nematode *Caenorhabditis elegans* have sizeable stretches of DNA which are identical to regions in the human genome [4]. The comparison of interspecies genomic differences (phylogenomics) [5] has not only provided a more meaningful juxtaposition of human beings among the other members of the animal kingdom, but also has increasingly become the focus of pharmaceutical companies whose goal it is to find new genes expressing protein products that can be modified for the benefit of the patient. The technological advances, such as automated sequencing of both DNA and protein, have led to the accumulation of vast amounts of data which are held in databases in the US, Europe and Japan. This ever-expanding repository of data is the basis for the computerized *in silico* research known as bioinformatics. Until now, in an attempt to associate diseases with abnormalities in single so-called candidate genes, the main thrust of research has focused on the 3 million or so single nucleotide polymorphisms (SNPs, pronounced 'snips') that constitute the major source of human diversity [6]. Having identified an association of a particular gene with a disease, it then becomes a straightforward, if somewhat laborious task of trying to find a substance that will alter the product of the gene, i.e. its respective protein, in a way that will modulate the disease process.

Genotyping, using polymerase chain reaction (PCR) based diagnostic tools has facilitated the rapid detection of a number of monogenic conditions whose early treatment may obviate potentially serious clinical consequences. Examples of this approach include screening for the BRCA1 and BRCA2 tumour suppressor genes in members of families with a history of breast cancer [7], screening for predisposition to some forms of heritable colonic cancer [8] and the detection of mutations in genes encoding cardiac ion channels, which can be the cause of sudden, unexpected death [9]. In certain conditions such as thromboembolic disease [10], breast cancer [11] and leukaemia [12], genetic screening can improve treatment strategies and prognosis calculation.

A further important aspect of molecular diagnostic screening involves the detection of SNPs in metabolic enzymes involved in drug detoxication, an area of genomics originally referred to as pharmacogenetics, a term coined half a century ago by Friedrich Vogel [13]. His work together with that of others such as Kalow [14] and Motulsky [15] laid the basis of the study of the influence of single gene abnormalities on drug disposition. The intervening years, however, have seen significant advances in the understanding of how genetics may influence not only the kinetics, but also the dynamics of drug action, e.g. the discovery of mutations in the genes for both receptors [16] and transporters [17]. In order to accommodate the wider implications of genome-based knowledge the term *pharmacogenomics* has been recently coined, and although there is as yet no clear distinction or definition of the two terms, it has been proposed that the term *pharmacogenomics* implies a study of the impact of not only single genes on medications but includes the study of all the genes which encode proteins involved in every aspect of a drug's metabolism, disposition and effect [18,19].

One of the first drugs whose variable metabolism caught the attention of researchers, and until now still widely used by anaesthetists, was suxamethonium. Subsequently, the clinical impact of the variable metabolism of a number of important drugs has been discovered. For example, thiopurine *S*-methyltransferase (TPMT) is an important enzyme involved in the phase II metabolism of a number of chemotherapeutic agents used in the treatment of

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Accepted for publication January 2004 EJA 1846

acute lymphatic leukaemia and a number of autoimmune conditions such as rheumatoid arthritis and myasthenia gravis, as well as in preventing the rejection of transplanted organs [20]. Substrates of TPMT include 6-mercaptopurine, thioguanine and azathioprine. Several studies have shown that patients with low enzyme activity are at high risk of serious, if not potentially fatal haematopoetic toxicity if treated with conventional doses of these drugs [21]. Around 10% of the population are heterozygous for the defective TPMT allele and are thus at intermediate risk for toxic side-effects, and one in 300 are at high risk, since they are homozygous for the mutant allele [22]. Genetic screening for the abnormal allele of TPMT has become a standard procedure in some centres prior to commencement of therapy. If TPMT deficiency is diagnosed, thiopurine medication is reduced by about 90% thus guaranteeing optimal therapy in all patients [23].

The cytochrome (CYP) P-450 enzymes, a superfamily of microsomal drug metabolizing enzymes, are the most important of the enzymes that catalyse phase I metabolism. These enzymes are classified according to their amino acid structure. Enzymes with more than 40% amino acid homology are grouped together in a single family. This is designated by a number, e.g. CYP2. If amino acid homology is greater than 55% the enzymes are grouped together in a subfamily, designated by a capital letter, e.g. CYP2E. A further letter designates the individual enzyme [24]. Of the 18 known CYP families that exist in human beings, those belonging to families 1–4 are those which are involved in the breakdown of drugs, pollutants and chemicals. Of these, families 1–3 are the most important. It has been estimated that 90% of the metabolism of drugs and other xenobiotics can be attributed to six main CYP enzymes: 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4 [25]. Of these, CYP1A2 is responsible for the metabolism of propanolol and theophylline, CYP2C9 breaks down warfarin, CYP2C19's substrates include the proton-pump inhibitor omeprazole as well as a number of anticonvulsants while CYP2E1 is responsible for the metabolism of volatile anaesthetic agents [26,27]. CYP3A4 is the most abundantly expressed phenotype and breaks down a large number of diverse substances. CYP2D6 is also extremely important and has a large number of substrates. These include the beta-blockers metoprolol and alprenolol, the class 1C antidysrhythmic propafenone, various antidepressants, antipsychotics such as droperidol, thioridazine and haloperidol, and 5HT3 inhibitors such as ondansetron and tropisetron. It is also responsible for the biological conversion of a number of analgesic pro-drugs to their active form. Some of these, such as codeine and tramadol, are used in anaesthesia. In the case of codeine, CYP2D6 converts the pro-drug into morphine [28].

Unfortunately, all CYP enzymes are coded for by genes with considerable polymorphism, i.e. they may contain one or more SNPs. Around 10% of Caucasians have SNPs in the CYP2D6 gene that result in defective biotransformation of the respective substrate. This leads to drug accumulation or, in the case of codeine, failure to form the active metabolite with corresponding inadequate analgesia. These patients are called poor metabolizers. There is also a subgroup of patients whose genome contains multiple active copies of the gene, resulting in rapid breakdown of codeine. This results in a high incidence of opioid related side-effects. These patients are referred to as ultra-rapid metabolizers [29]. Until now it has been impossible, without resorting to expensive and time-consuming screening, to determine which patients will respond to codeine-based medications. Recently, however, a simple gene-testing device has been developed which facilitates the rapid and accurate determination of an individual's genotype. The 'Amplichip CYP450' (Roche®, Basel, Switzerland), which will appear on the market later in 2004 is essentially a microarray developed to determine the status of two genes: CYP2D6 and CYP2C19 [30]. This simple chip represents a breakthrough in pharmacogenomic testing and allows rapid detection of those patients who will react abnormally to a wide range of important therapeutic substances including analgesics. The Amplichip® is able to probe the patient's DNA for a wide range of polymorphisms including all the common alleles that are responsible for poor metabolizer status as well as those associated with the ultra-rapid phenotype.

DNA microarray technology is a sophisticated but conceptually simple and cost-effective means of monitoring the expression levels of thousands of different genes simultaneously. Synthetic, complementary DNA (cDNA) fragments, representing different gene clones are spotted at high density onto a rectangular grid on a solid support, such as glass or nylon, using miniaturized robotic techniques. Each cDNA spot can then be probed against a fluorescently or radioactively labelled DNA target. The intensity of the resulting signal corresponds to the amount of the respective DNA in the target or sample. Thousands of gene samples can be tested in a single experiment and indeed following the completion of the human genome project it is anticipated that within the foreseeable future the entire human genome may be used as a probe [31]. An alternative microarray technology pioneered by the American biotech company Affymetrix and used in the Amplichip® synthesizes a series of oligonucleotides *in situ*, using a technique based upon photolithography and is used extensively in the microchip industry. The sequences are derived from those held in gene databases.

The clinical and diagnostic implications of microarray technology are far reaching. Until now, prohibitive costs have restricted the use of microarrays primarily to institutions, which enjoy the luxury of generous research funding, and to pharmaceutical companies. In the arena of drug discovery, they can be used both as a means of identifying new targets, as well as to examine abnormal patterns of gene expression following exposure to a chemical or drug, a field known as *toxicogenomics*. The underlying principle of toxicogenomics is the hypothesis that every adverse drug reaction is the result of, or is accompanied by, an abnormal gene expression. Indeed, the change in gene programming occurs far earlier than the clinical manifestation of a drug reaction [32]. In addition, considerable effort is being made to characterize the interspecies variation in gene expression which is a vital step before acquired animal data can be extrapolated to human beings (pharmacophylogenomics) [33]. It is also being used to characterize gene profiles in complex conditions such as sepsis [34].

Now, for the first time, the advantages of microarray technology have been made available to clinicians enabling them to optimize therapy for patients receiving a variety of drugs, including analgesics, antidepressants and antipsychotics in a number of diverse clinical areas. At present, the Amplichip® detects polymorphisms in only two genes. It may be anticipated that in the foreseeable future the scope of commercially available gene chips will expand greatly, not only allowing rapid diagnosis of abnormalities in drug metabolizing enzymes, but also allowing clinicians to pre-empt rare, but potentially life threatening, conditions such as malignant hyperpyrexia which is linked to an abnormality of the ryanodine gene [35]. At present there is less than optimal knowledge concerning optimization of treatment in patients with complex polygenic conditions such as hypertension and diabetes, although in the case of asthma it is known that certain patients with aberrant alleles of both the beta-2-adrenergic receptor and the 5-lipoxygenase gene (ALOX5 gene), which controls leukotriene synthesis, have altered responses to beta-2-stimulants [36] and leukotriene receptor antagonists [37], respectively. It might similarly be anticipated that in the future routine pre-operative assessment will include genetic screening of appropriate receptors and pathways to help optimize therapy where necessary. Slowly the dream of *pharmacogenomics*, to provide tailor-made therapy for all, is becoming a reality.

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https://doi.org/10.1017/S026502150400701X Published online by Cambridge University Press