Ajeet Singh
MBBS(Melb), MPsych(Melb),
FRANZCP, is Consultant
Psychiatrist, The Geelong
Clinic, Geelong, Victoria.
ajeetsingh@dodo.com.au

Jon Emery
MA, MBBCh, MRCGP, FRACGP,
DPhil, is Professor, Discipline
of General Practice, University
of Western Australia.

Pharmacogenomics
The potential of genetically guided prescribing

BACKGROUND
Matching patients to effective tolerable medications can take months of trial and error. Genetic factors can strongly influence drug response. One of the potential clinical applications of the Human Genome Project is prediction of medication response – pharmacogenomics.

OBJECTIVE
This article reviews the emerging field of pharmacogenomics and its clinical potential using the example of antidepressant and anticoagulant medication prescribing.

DISCUSSION
Growing evidence suggests genomic profiling for common variants in cytochrome P450 enzymes and transport systems involving serotonin, noradrenaline and the blood-brain barrier could inform antidepressant prescribing. Similarly, genetic testing for variants in cytochrome CYP2C9 and vitamin K epoxide reductase could inform decisions about prescribing warfarin. Large clinical trials to determine the clinical utility of this approach are required before pharmacogenomics enters routine clinical practice.

Over 20 000 genes have been identified and sequenced since the Human Genome Project was commenced in 1990. Interestingly, we differ in only one base pair per 1000 genes. This 0.1% genetic variation holds keys to disease susceptibility and differential response to drugs.

Single base pair differences (single nucleotide polymorphisms or SNPs) are the commonest form of genetic variation between people. Mounting evidence suggests certain SNPs are associated with different pharmacokinetic and pharmacodynamic responses to a range of commonly prescribed drugs.

With some 3 billion nucleotides in the human genome, 0.1% variation between individuals translates to 3 million SNPs. Previously, only one gene at a time could be investigated, making rapid genotyping impossible. But the development of micro-array technology opens the possibility of rapid and increasingly cost effective patient genotyping. The practical issue is whether such testing has clinical utility.

Many authorities believe the first major clinical application of the Human Genome Project will be genetically guided prescribing – pharmacogenomics.[1] This article discusses the concept of pharmacogenomics in the context of two clinical examples: antidepressant and warfarin prescribing.

Pharmacogenomics – antidepressants

Fifty years ago the first antidepressants came in to use. Subsequently their safety and tolerability has been improved, but efficacy remains little changed.[2] Genetic prediction of antidepressant response has potential to inform the choice of agent and tailor dose to improve speed of response and reduce adverse effects.[3–6]

One of the most frustrating and problematic aspects of the pharmacological management of depression is the 'hit and miss' nature of response. It can take months of trial and error before an effective medication is found for a given individual. During this time patients are exposed to the morbidity and potential mortality from this common and disabling illness.[7,8]

Advances in our understanding of the pathophysiology of depression, including the molecular biology of limbic-cortical dysregulation, are beginning to inform individual variation in response to antidepressants.[9] Genetic variation affects both the metabolism of drugs (pharmacokinetics) and drug response (pharmacodynamics).

Genetic variation in the pharmacokinetics of antidepressants

The alimentary tract, liver, and blood brain barrier are the main pharmacokinetic hurdles for antidepressants to reach

their site of action in the limbic-cortical tracts.[10,11] While most second generation antidepressants are well tolerated, some patients experience significant side effects at usual starting doses. Other patients need high doses to achieve efficacy with initial underdosing delaying response. These problems are routinely observed in clinical practice.[12] For each individual an effective and tolerable dose of medication could potentially be predicted from the polymorphism profile of key enzymes and transporter systems involved in the pharmacokinetics of the antidepressant.[13]

Differing frequencies of side effects and efficacy have been noticed between individuals of different ethnic groups.[3] Much of this variation is due to different polymorphism frequencies for the hepatic enzymes that metabolise drugs. The most well studied system is the cytochrome P450 system. Some P450 enzymes are found in the alimentary tract wall; the majority in hepatocytes. These enzymes (particularly cytochrome P450 2D6 and 2C19) are involved with the inactivation of many antidepressants and other medications. Slow, rapid, and ultra rapid metabolisers have been identified from different SNP profiles.[14]

Patients with genotypes associated with ultra rapid metabolism at P450 enzymes will have lower serum levels of antidepressants at standard starting dose than patients with slower metabolism. Such patients have lower rates of side effects but need higher doses for efficacy (see *Case study 1*). In contrast, slow metabolisers are more likely to experience side effects at 'standard' doses (*Table 1*).

The blood-brain barrier (BBB) has active transporter systems that keep medications and other substances from reaching the cerebrospinal fluid (CSF). The ABC family of transporters actively pumps many agents out of the CSF.[15] MDR1 is one of the better characterised BBB pump systems. Polymorphisms in MDR1 have been associated with differential rates of antidepressant entry to CSF.[16] Clinical relevance to dosing of antidepressants has yet to be demonstrated. Certain individuals may have more rapid pump systems and a less medication permeable BBB. Such patients would tend to have fewer central side effects but show reduced response to antidepressants even at high medication serum levels. Such patients require higher than average antidepressant dose to respond. This emerging area of BBB pharmacogenomics may explain why some patients have fewer central side effects and poorer response. Theoretically, MDR1 and P450 genotyping could enable determination of who requires a large dose and who does not from treatment outset. This could reduce side effects and speed rates of response by getting the dose tailored right from the start (*Table 1*). Large clinical studies are required to confirm the role of such BBB genotyping in clinical practice.

Case study 1

A man aged 32 years, divorced and on sickness benefits, is being treated with venlafaxine for major depression. His response to the current maximum recommended dose of 225 mg/day is only partial after 1 month on that dose. He re-presents with ongoing poor energy and confidence. Genotyping demonstrates he carries multiple CYP2D6*1 variants associated with ultra fast metabolism. On this basis dose is increased (gradually with side effect monitoring) to 375 mg/day. The patient recovers to his premorbid level of functioning, copes with job seeking, and soon finds work.

Genetic variation in the pharmacodynamics of antidepressants

Serotonin, noradrenaline, dopamine, melatonin, corticotropin releasing hormone, glutamate, gama amino butyric acid, brain derived neurotrophic factors, substance P and other centrally acting substances are essential to limbic-cortical functioning.[9] Various patterns of dysregulation in these systems can be precipitated by chronic stress. Current antidepressants act primarily on the monoamines serotonin, noradrenaline, and dopamine.[10] Physiological factors are important determiners of why one patient responds to one antidepressant and not another. Recent findings suggest that in the future genomic tests may be used to determine which type of antidepressant patients are most likely to respond to.[17,18]

In recent years, associations have been demonstrated between serotonin transport gene polymorphisms and susceptibility to depression.[19,20] It has also emerged that there is genetic variation in noradrenaline dysregulation in the limbic-cortical system.[17,18] Early evidence suggest that genotyping for polymorphisms in the serotonin transporter promoter region gene 5-HTTLPR and the norepinephrine transporter gene NET polymorphisms may help inform clinical choice of noradrenergic or serotonergic antidepressants[18] (see *Case study 2*). It is likely that further pharmacodynamically relevant genes will be identified to match patients to the most likely effective antidepressant from the outset of pharmacotherapy.

Pharmacogenomics of warfarin

The vitamin K antagonist warfarin remains the most widely prescribed oral anticoagulant for thromboembolic disorders. Its narrow therapeutic window and over 10-fold interindividual therapeutic dose variability causes problems with dosing regimens and significant risk of bleeding.[21]

Warfarin is a mixture of S- and R-enantiomers with the S-enantiomers having most of the anticoagulant effect. Cytochrome P450 CYP2C9 is largely responsible for hydroxylation inactivation of the S-enantiomer. Warfarin

Table 1. Potential matching of genotype to antidepressant dosing			
P450 2D6 & 2C19 genotypes → MDR1 genotype ↓	**Rapid metaboliser**	**Intermediate metaboliser**	**Slow metaboliser**
Low BBB permeability	Highest range dose	High dose	Intermediate dose
Intermediate BBB permeability	High dose	Intermediate dose	Low dose
High blood-brain barrier permeability	Intermediate dose	Low dose	Lowest range dose

inhibits vitamin K epoxide reductase. This hepatocyte endoplasmic reticulum enzyme is encoded by the recently identified gene VKORC1 (vitamin K epoxide reductase complex 1). The enzyme recycles reduced vitamin K which is required for activation of clotting factors II, VII, IX and X. Vitamin K epoxide reductase inhibition by warfarin decreases the level of reduced vitamin K, therefore less activated clotting proteins are synthesised leading to the anticoagulant effect of warfarin. Different polymorphisms of CYP2C9 and VKORC1 have different functional activity, and this helps explain why some patients require more and some less warfarin to achieve target INRs.

Possession of CYP2C9*2 or CYP2C9*3 variant alleles is associated with higher serum warfarin levels, greater risk of bleeding, and a significantly lower mean warfarin dose needed to obtain therapeutic anticoagulation.[22]

Polymorphisms of the VKORC1 gene are also associated with warfarin dose requirements.[21,23,24] Low dose requiring and a high dose requiring single nucleotide polymorphisms of the VKORC1 gene have been identified. These VKORC1 polymorphisms account for an approximately 3-fold greater effect on interindividual variability in dose requirements than CYP2C9 polymorphisms. Individuals at increased risk of either overanticoagulation and bleeding or underanticoagulation and thrombosis could be predicted through CYP2C9 and VKORC1 genotyping before initiating treatment. Those at greater risk of bleeding could be offered alternative antithrombotic medication, particularly in the case of stroke prevention in atrial fibrillation. Different initiation and monitoring regimens might also be informed by the CYP2C9 and VKORC1 genotype profile.

It should be recognised that nongenomic factors such as age, body mass index (BMI), dietary vitamin K intake, comorbid disease and drug interactions also influence warfarin dosing.[25] Combining genomic and nongenomic factors to tailor dose and monitor regimen before initiating treatment reduces the potentially fatal adverse events from both under- and over-anticoagulation (see *Case study 3*).

Dawn of a new era

While we have presented examples of how pharmacogenomics has significant potential to improve tailored prescribing in general practice, further evidence to demonstrate clinical utility is required before rushing into wide scale patient genotyping. Given the greater tolerability and lower toxicity of second generation antidepressants, it is likely that pharmacokinetic factors such as cytochrome P450 profile will have only limited clinical value. General practitioners and psychiatrists currently take the pragmatic approach and titrate up to the maximum tolerable dose if patients are not responding. Doubts about the true clinical utility of P450 genotyping and antidepressant prescribing have recently arisen.[4–6] Similarly, what is the relative cost effectiveness of genotyping before prescribing warfarin when we already have a cheap and effective phenotypic marker of drug metabolism, the INR?

Not all scientific advances bear clinically useful fruit. However, the confluence of emerging clinical evidence suggests antidepressant and anticoagulant pharmacogenomics may become useful clinical tools in informing drug selection and dosing to maximise early response and reduce adverse effects. P450 testing is already commercially available in Australia and is becoming

Case study 2

An anxious woman, 26 years of age, re-presents with worsening features of major depression despite focused psychological counselling. She is started on a selective serotonin reuptake inhibitor (SSRI). Despite a high dose she fails to respond. Genotyping demonstrates her to carry the NET G1287A GG genotype (associated with better response to noradrenaline reuptake inhibitors).[21] She is switched to reboxetine (a noradrenaline reuptake inhibitor) and on follow up 2 weeks later is less anxious, coping better, and her depressive symptoms are markedly reduced.

Case study 3

An active widow, 81 years of age, has diabetes. Atrial fibrillation (AF) is detected on routine medical review. Her diabetes is well controlled and no secondary causes for her AF are identified. Genotyping demonstrates she has the CYP2C9*3 polymorphism associated with higher risk of bleeding on warfarin. In discussion with her GP she decides to take aspirin for stroke prevention rather than warfarin.

less costly. Genetically guided prescribing tailored to the pharmacokinetic and pharmacodynamic patient genotype looks set to enable optimal medication and dose selection in advance. Such prediction of medication response could, in theory, help reduce the burden of crippling and prevalent disorders – time will tell.

Summary of important points

- Interindividual variation in medication response is often related to genetic differences such as SNPs. In some circumstances these can now be predicted through genotyping and potentially guide more effective tolerable prescribing.
- Both antidepressant prescribing and warfarin dosing is currently somewhat 'hit and miss'. Emerging research suggest that genotyping may assist in prescribing these drugs and provides a clinically salient example of the potential scope of pharmacogenomics.
- Cytochrome P450, BBB, serotonergic, and noradrenergic system polymorphisms help predict antidepressant response patterns, and may assist agent and dose selection from outset of pharmacotherapy.
- Pharmacogenomic profiling of cytochrome P450 and vitamin K epoxide reductase polymorphisms may assist with warfarin dosing and risk-benefit analysis from outset of treatment.
- Additional large scale clinical evidence is needed with demonstration of cost effectiveness before pharmacogenomics become a helpful part of routine clinical practice.

Conflict of interest: none declared.

References

1. Lee M. The pharmacogenetics of antidepressant treatments for depressive disorders. Drug Dev Res 2005;65:170–8.
2. Mulrow CD, MD, Williams JW, Chiquette E, et al. Efficacy of newer medications for treating depression in primary care patients. Am J Med 2000;108:154–64.
3. De Leon J, Armstrong S, Cozza K. Clinical guidelines for psychiatrists for the use of pharmacogenetic testing for CYP450 2D6 and CYP450 2C19. Psychosomatics 2006;47:75–85.
4. Perlis RH. Cytochrom P450 genotyping and antidepressants. BMJ 2007;334:759.
5. Lee MS. Could pharmacogenetics play a role as predictor in treatment of depressive disorders? World J Biol Psychiatry 2007;8:2–3.
6. Thompson CA. Too soon to judge value of pharmacogenetic testing in selecting antidepressant. Am J Health Syst Pharm 2007;64:453.
7. Mathers C, Vos T, Stephenson C, et al. The burden of disease and injury in Australia. Bull World Health Organ 2001;79:1076–84.
8. Murray C, Lopez A, Mathers C, et al. The global burden of disease. Geneva: World Health Organisation, 2001.
9. Bondy D. Pathophysiology of depression and mechanisms of treatment. Dialogues Clin Neurosci 2002;4:7–20.
10. Richelson E. Pharmacology of antidepressants. Mayo Clinic Proceedings. 2001;76:511–27.
11. Poolsup N, Po A, Knight T. Pharmacogenetics and psychopharmacotherapy. J Clin Pharm Therapy 2000;25:197–220.
12. Doris A, Ebmeier K, Shajahan. Depressive illness. Lancet 1999;354:1369–75.
13. Nierenberg A. Prediction of response to antidepressants: general principals and clinical implications. Psychiatric Clin N Am 2003;26:345–52.
14. Lee MS. Could pharmacogenetics play a role as predictor in treatment of depressive disorders? World J Biol Psych 2007;8:2–3.
15. Hung C, Tai J, Lin C, et al. Complex haplotypic effects of the ABCB1 gene on epilepsy treatment response. Pharmacogenomics 2005;6:411–7.
16. Eichelbaum M, Fromm M, Schwab M. Clinical aspects of the MDR1 (ABCB1) gene polymorphism. Ther Drug Monit 2004;26:180–5.
17. Yoshida K, Takahashi H, Higuchi H, et al. Prediction of antidepressant response to milnacipran by norepinephrine transporter gene polymorphisms. Am J Psychiatry 2004;161:1575–80.
18. Kim H, Lim S, Kim S, et al. Monoamine transporter gene polymorphisms and antidepressant response in Koreans with late life depression. JAMA 2006;296:1609–18.
19. Caspi A, Sugden K, Moffitt T, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386–9.
20. Marcus R, Munafò A, Taane G, et al. Neuroticism mediates the association of the serotonin transporter gene with lifetime major depression. Neuropsychobiology 2006;53:1–8.
21. Tong Y, Toshiyuki M. Warfarin dose and the pharmacogenomics of CYP2C9 and VKORC1 – rationale and perspectives. Thromb Res 2007;120:1–10.
22. Sanderson S, Emery J, Higgins J. CYP2C9 gene variants, drug dose, and bleeding risk in warfarin treated patients: a HuGEnet[TM] systematic review and meta-analysis. Genet Med 2005;7:97–104.
23. Schalekamp T, Brasse BP, Roijers JFM, et al. VKORC1 and CYP2C9 genotypes and acenocoumarol anticoagulation status: interaction between both genotypes affects overanticoagulation. Clin Pharmacol Ther 2006;80:13–22.
24. Rieder MJ;Reiner AP, Gage BF, et al. Effect of VKORC1 Haplotypes on Transcriptional Regulation and Warfarin Dose. N Engl J Med 2005;352:2285–93.
25. Kamili F, Pirmohamed M. The future prospects of pharmacogenetics in oral anticoagulation therapy. Br J Clin Pharm 2006;61:746–51.

afp CORRESPONDENCE email: afp@racgp.org.au