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Pharmacogenetic Testing

Advanced insights for optimised drug treatment outcomes

Pharmacogenetic Testing

By Associate Professor Mirette Saad

Pharmacogenetics (PGx), an important part of precision medicine, is the study of how genetic variability influences drug treatment outcomes. Recommended by Guidelines, many medications currently prescribed have pharmacogenetic data to support appropriate dosing or selection. Like all diagnostic tests, pharmacotherapeutic genotyping is one of multiple pieces of information that clinicians should consider when making their therapeutic choice for each patient.

Clinical Labs offers a comprehensive range of pharmacogenetic testing in order to provide Clinicians and Healthcare providers with important information to help decide on the most appropriate treatment for each individual, particularly in areas such as mental health, pain management, cardiology and oncology.

PGx test utility

Implementation of clinical pharmacogenetics, allele function and inferred phenotypes is a crucial step toward optimum patients' health. Identifying responders and non-responders to medications can reduce morbidity, avoid adverse events and optimise drug dosing.

Literature has shown that a large number of people are injured or die each year in hospitals from adverse drug events (ADEs), costing millions of dollars in healthcare costs each year. The field of genomic medicine presents one potential solution to reduce healthcare costs associated with ADEs and poor response to pharmacotherapy.

> *"The field of pharmacogenetics involves using a patient's genetic makeup in combination with other clinical information to create a personalised medication regimen with greater efficacy and safety for the individual patient."*

PGx guidelines

Evidence-based guidelines with pharmacotherapeutic recommendations for combinations of specific drugs and genotypes or predicted phenotypes are essential for implementing acquired pharmacogenetics knowledge in daily clinical practice.

The Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) have been developing guidelines for more than a decade (Swen *et al.* 2011a; Caudle *et al.* 2017).

Recommendations are preferably made available at the time of drug prescribing and dispensing for a patient with a genotype that requires an action, such as a dose reduction (Swen *et al.* 2011a; Deneer and van Schaik, 2013). When to order the test?

Physicians may order the pharmacogenetic testing per drug at the point of care, or an alternative approach to ordering is the use of pre-emptive testing, perhaps as part of an annual exam in young adults or even children that require multiple treatments. As a result of the increasing number of drugs with pharmacogenetic data, the pre-emptive use of testing could significantly optimise drug outcomes (Schildcrout *et al.* 2012).

Regardless of when ordered (at time of treatment or prior), due to the continuing decline in the costs of genomic testing technologies, a broad-based pharmacogenetic screen may yield the greatest cost savings.

The *cytochrome P450 (CYP450)* and differences in drug metabolism

A family of enzymes (Figure 1), catalyses the metabolism of many drugs and xenobiotics. The genes that code for *cytochrome P450* enzymes are highly polymorphic, which can significantly affect drug metabolism in certain individuals. Differences in drug metabolism due to *CYP450* gene variants influence plasma levels of both the active drug and its metabolites.

For example, *CYP2D6, CYP2C19* and *CYP2C9* are responsible for the metabolism of a large number of commonly prescribed drugs, including warfarin, analgesics, clopidogrel, codeine, tamoxifen, some antidepressants, statins, proton pump inhibitors (PPIs) and anti-emetics (See Table 1). *CYP3A5* genotype results can be used to guide dosing of tacrolimus in organ transplant patients (Birdwell *et al*. 2015).

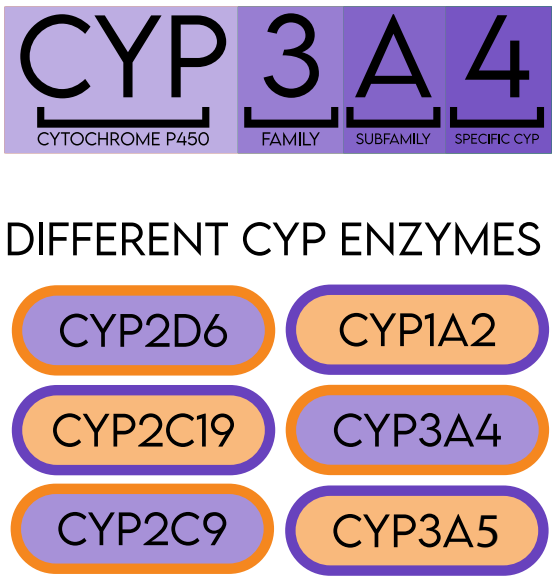

Figure 1. CYP Cytochrome P450 enzyme nomenclature and examples chart.

CYP2D6

CYP2D6 is the primary enzyme responsible for the metabolism of many commonly-used medications especially in mental health, oncology (tamoxifen and 5-HT3 receptor antagonists) (Goetz *et al*. 2018) and pain management (Crews *et al*. 2021) (Table 1 & Figure 2). *CYP2D6* is highly polymorphic with over 130 identified allelic variants and sub-variants identified (www.PharmVar.org; *CYP2D6* Allele Definition).

CYP2D6 alleles have been extensively studied in multiple geographically, racially, and ethnically diverse groups, and significant differences in allele frequencies have been observed. It is important to note that variation in *CYP2D6* may have implications for many therapies that may not be listed on this report (Gaedigk *et al*. 2017).

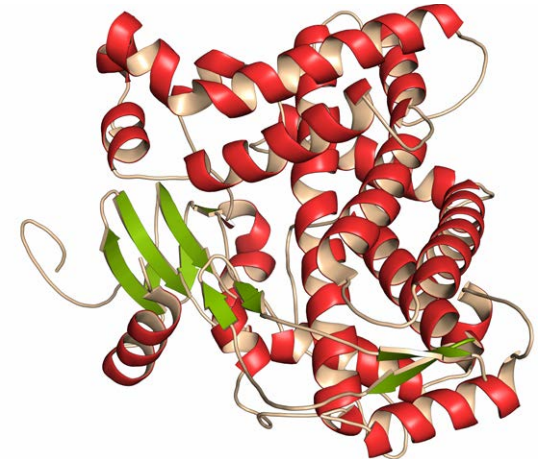

Figure 2. Cytochrome P450 (CYP2D6) liver enzyme in complex with a drug.

CYP2C19

The hepatic *CYP2C19* enzyme contributes to the metabolism of a large number of clinically relevant drugs such as antidepressants, benzodiazepines, mephenytoin, some proton pump inhibitors (Lima *et al*. 2021), and clopidogrel (Scott *et al.* 2013) and anti-fungal medication (voriconazole) (Moriyama *et al.* 2017) (Table 1). Like many other *CYP450* superfamily members, the *CYP2C19* gene is highly polymorphic, with >25 known variant alleles.

CYP2C9

Variants in the *CYP2C9* genes modify the rate at which some medications are metabolised. When considering antidepressant therapy such as tricyclic anti-depressants (TCAs), *CYP2C9* test is often combined with analysis of the CYP2C19 and *CYP2D6* genes (Attia *et al.* 2014 and Hicks *et al.* 2017). When considering warfarin therapy, this test is often combined with analysis of *VKORC1.*

VKORC1 and *CYP2C9* and warfarin

Warfarin is one of the most commonly prescribed medications worldwide, used for many indications including prophylaxis and treatment of thromboembolic disorders, atrial fibrillation, or cardiac valve replacement, and systemic embolism after myocardial infarction (MI). Approved in the US in 1954, the high efficacy of warfarin is challenged by the high risk of ADEs due to its narrow therapeutic window, requiring careful monitoring and strict compliance.

While *CYP2C9* is predominantly involved in the metabolism of warfarin subtypes; *VKORC1* is the molecular target of the drug. In 2017, an international collaboration published an updated landmark paper defining appropriate warfarin doses based on a validated dosing algorithm of clinical biomarkers and *VKORC1/CYP2C9* genotypes (Johnson *et al*. 2017).

SLCO1B1 and statins

SLCO1B1 gene testing is clinically important in clearance of statins, especially simvastatin. Myopathy is reported in poor metabolisers of this gene. Alternative lipid lowering statins can be prescribed in lower doses such as atorvastatin, pravastatin and rosuvastatin (Ramsey *et al*. 2014).

Genetic variations can render some medications ineffective or toxic

Pharmacogenetic variants result in four distinct phenotypes: normal metabolisers (NMs), intermediate metabolisers (IMs), poor metabolisers (PMs), and ultrarapid metabolisers (UMs) which provides guidance to drug dosing and selection.

Overall, wild-type alleles are usually associated with functional enzyme-mediated metabolism. *Ultrarapid metabolisers* may not achieve therapeutic plasma levels due to decreased trough drug concentrations, whereas *poor metabolisers* treated with drugs that are metabolised by these enzymes are at increased risk for prolonged therapeutic effect or toxicity due to increased trough levels of therapeutic drugs.

Some anti-psychotic and SSRI medications can be contraindicated in intermediate *CYP2D6* metabolisers due to increased risk of adverse effects and so alternative agents must be prescribed.

CYP2D6 ultrarapid metabolisers treated with codeine exhibit symptoms of extreme sleepiness, confusion or shallow breathing; the lowest possible dose should be prescribed to these patients. Meanwhile, patients that are *CYP2D6 poor metabolisers* will not achieve sufficient pain control due to their inability to convert the drug to its active form of morphine (Crews *et al.* 2021).

CYP2C19 ultrarapid metabolisers should be prescribed alternative therapeutic agents other than benzodiazepines, such as citalopram (Celexa) and escitalopram (Lexapro) and TCAs such as impiramine (Tofranil) and clomipramine (Anafranil) due to possible decreases in the efficacy of these medications.

Pharmacogenetic markers in oncology

In addition to *RAS, BRAF, EGFR, ERBB2 (HER2), PK3CA* and *KIT* mutation and *PD-1, ROS, ALK* and *BCR-ABL* fusion genes, other genetic pharmacogenetic biomarkers play a role in patients' responses to oncology therapy.

UDP-glucuronosyltransferase gene (UGT1A1)

UGT1A1 is involved in the metabolism of *irinotecan* (Figure 3), a topoisomerase I inhibitor. *UGT1A1* gene polymorphism is associated with toxicity and clinical efficacy of irinotecan-based chemotherapy in patients with advanced solid tumours including colorectal, rectal and lung cancer (Fujii *et al.* 2019).

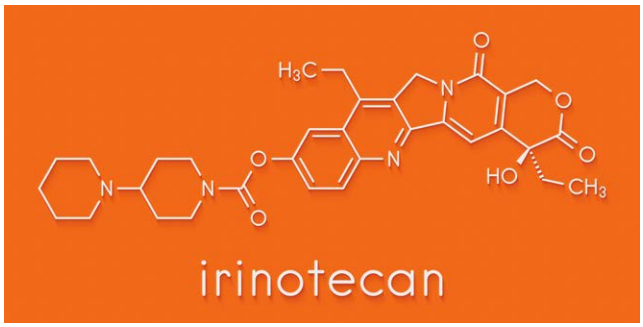

Figure 3. Irinotecan cancer chemotherapy drug molecule.

Thiopurine methyltransferase (TPMT)

TPMT is the primary enzyme responsible for thiopurine drugs (azathioprine, 6-mercaptopurine and 6-thioguanine) metabolism. These drugs are converted in the body to thioguanine nucleotides (TGNs).

Thiopurine therapy targets the replicating cells without overly harming normal cells. Several studies have established Single Nucleotide Polymorphisms (SNPs) in the *TPMT* gene that may lead to enzyme inactivity and therefore haematopoietic toxicity due to thiopurine drugs. It is recommended that physicians order *TPMT* genotyping before prescribing thiopurines to avoid bone marrow toxicity and consequent neutropenia (Relling *et al*. 2018).

Dihydropyrimidine dehydrogenase gene (DPYD)

DPD stands for dihydropyrimidine dehydrogenase, an enzyme made by the liver that breaks down uracil and thymine. The molecules created when pyrimidines are broken down (5,6-dihydrouracil and 5,6-dihydrothymine) are excreted by the body or used in other cellular processes. *DPYD* gene mutations result in excess quantities of the breakdown molecules in the blood, urine, and cerebrospinal fluid.

Mutations in the *DPYD* gene also interfere with the breakdown of drugs with structures similar to the pyrimidines, such as the cancer drugs 5-fluorouracil and capecitabine (two common chemotherapy drugs used as a treatment for a number of different cancers). As a result, these drugs accumulate in the body and cause the severe reactions and neurological manifestations as a result of DPD deficiency (Amstutz *et al*. 2017).

Conclusion

The incorporation of genetic information obtained from pharmacogenetic testing holds substantial promise to improve therapeutic decision making through improved efficacy and reduced adverse events. Considerations for clinical implementation, such as optimal laboratory workflows, electronic health record integration, and stakeholder engagement, as well as provider education, are crucial for patients' health.

Pharmacogenetic (PGx) test list at Australian Clinical Labs

Our comprehensive pharmacogenetic tests can detect polymorphisms in genes coding for drug metabolising enzymes that predispose individuals to metabolising drugs inadequately.

Gene panels offered:

- ***Cytochrome P450 Comprehensive Gene Panel including****:

CYP2D6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP1A2, SLCO1B1 and *VKORC1*

Single gene test:

- *TPMT (Medicare rebate)*
- *DPYD*
- *UGT1A1*
- *CYP2D6*
- *CYPC9*
- *CY2C19*

*Please note that the panel Cytochrome P450 Genes can beordered separately or together (CYP2D6, CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP1A2, SLCO1B1 and VKORC1).

When to order: Before commencing therapy, with adverse reaction or resistance.

How to order: Fill out our routine Clinical Labs testing request form, list the gene required or group of genes and prescribed medications if available.

Turnaround time: Results will be available after 7-10 business days from the sample receipt date.

Specimen required: 2x EDTA blood samples.

Report: With the Cytochrome P450 Comprehensive Gene Panel you will receive a comprehensive report* that will indicate the genotype and the predicted phenotypes, such as the metaboliser status along with potential drug-gene interaction and guidelines' recommendations. Please specify any medications of interest if you want them to be included in the report. For individual genes, only genotyping/phenotyping will be reported.

Test cost: Apart from the TPMT gene, CYP450 Variants are non-Medicare (an out-of-pocket fee applies).

References:

Amstutz *et al*. 2017. Clin Pharmacol Ther

Attia *et al*. 2014. Chem Pharm Bull

Birdwell *et al*. 2015. Clin Pharmacol Ther

Caudle *et al*. 2017. Clin Pharmacol Ther

CPIC 2023, Guideline for CYP2D6, CYP2C19 and SSRI Antidepressants

CPIC 2022, Guideline for SCLO1B1, CYP2C9 and Statins

CPIC 2022, Guideline for CYP2C19 and Clopidogrel Dosing

CPIC 2020 Guideline for CYP2C19 and Proton Pump Inhibitor Dosing

CPIC 2020 Guideline for CYP2C9 and NSAID Therapy

CPIC 2019 Guideline for CYP2D6 and Atomoxetine

Crews *et al*. 2021. Clin Pharmacol Ther

Deneer and van Schaik, 2013. Pharmacogenomics

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Guidelines – CPIC (cpicpgx.org)

Hicks *et al*. 2017. Clin Pharmacol Ther

Johnson *et al*. 2017. Clin Pharmacol Ther

Klein *et al*. 2009. N Engl J Med

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Moriyama *et al*. 2017. Clin Pharmacol Ther

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Schildcrout *et al*. 2012. Clin Pharmacol Ther

Scott *et al*. 2013. Clin Pharmacol Ther

Swen *et al*. 2011a. Clin Pharmacol Ther

Table 1: Selection guide of genes tested based on medications of use

GENE	Type of Metabolised Medication	Metabolised Drugs		Medicare Rebate
CYP2D6	Anti-Psychotics Anti-Depressants Pain Management Oncology Cardiology Neurology Urology Others	**Anti-Psychotics** Aripiprazole Brexpiprazole Chlorpromazine Haloperidol Risperidone Zuclopenthixol **SSRI** Citalopram Escitalopram Paroxetine Sertraline **TCAs** Amitriptyline Clomipramine Desipramine Doxepin Imipramine Nortriptyline Trimipramine **Oncology** Tamoxifen Gefitinib **Cardiology** Carvedilol Flecainide Metoprolol	**Opioids and Pain Management** Codeine Dihydrocodeine Morphine Naltrexone Oxycodone Tramadol **Neurology, Anti-ADHD and Anti-Dementia** Atomoxetine Dextroamphetamine Lisdexamfetamine Donepezil Galantamine **Urology Medication** Darifenacin Mirabegron Tamsulosin Tolterodine **Others** Metoclopramide Ondansetron	N/A
CYP2C9	Pain Management Anti-Coagulant (Warfarin)	**NSAIDs** Celecoxib Flurbiprofen Ibuprofen Meloxicam Piroxicam	**Neurology** Phenytoin **Anti-Coagulant** Warfarin	N/A

GENE	Type of Metabolised Medication	Metabolised Drugs		Medicare Rebate
CYP2C19	PPIs Anti-Platelets (Clopidogrel) Anti-Depressants Neurology	**PPIs:** Esomeprazole (Nexium) Lansoprazole Omeprazole (Losec) Pantoprazole Rabeprazole **Anti-Platelets** Clopidogrel **SSRI** Citalopram Escitalopram Fluoxetine (Prozac) Fluvoxamine Paroxetine Sertraline (Zoloft) **Anti-Psychotics** Clozapine	**TCAs** Amitriptyline Clomipramine Desipramine Doxepin Imipramine Nortriptyline Trimipramine **Benzodiazepines (Anxiolytics)** Clobazam Diazepam (Valium) **MAOIs** Moclobemide **Neurology** Phenytoin	N/A
SLCO1B1	Lipid Lowering Medications (Cardiology)	Simvastatin Pravastatin		N/A
CYP1A2	Anti-Psychotics Anti-Depressants	Clozapine Duloxetine Olanzapine		N/A
CYP3A4	Psychiatric Medications Statins Organ Transplant Pain Management Others	Atorvastatin Codeine Diazepam Quetiapine Simvastatin Tacrolimus		N/A
CYP3A5 (and CYP3A4)	Organ Transplant	Tacrolimus		N/A
VKORC1	Anti-Coagulant	Warfarin		N/A
TPMT	Oncology	Azathioprine Cisplatin Mercaptopurine Thioguanine		N/A
DPYD	Oncology	Capecitabine 5-Fluorouracil Tegafur		N/A
UGTA1	Oncology	Atazanavir Belinostat Binimetinib Irinotecan Nilotinib Pazopanib		

Please note that this is a guide for gene selection. Some specific medications may not be reported if they are listed under a drug class that is metabolised by the relevant gene. Content in this table is correct as of 06.06.23.

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