

The INFINITI™ Analyzer

The INFINITI™ Analyzer is an automated microarray platform which is capable of performing low and medium density multiplex assays.

Methodology to Identify Genetic Variants

Sample

- Whole blood with EDTA as an anticoagulant. Blood may be sent at ambient temperature or frozen.
- DNA will be extracted upon receiving the samples.
- Extracted DNA may be tested immediately or can be frozen at -80°C and used at a later date. DNA samples will be used for Infiniti PCR. Samples may be batched and processed.

Infiniti CYP assays

- All assays are pre-standardized by Autogenomics and are ready to use.
- PCR will be performed as per the instructions of the Infiniti application. Note: PCR run time varies from 2 – 4 hrs for different assays.
- The PCR products will be further processed in the Infiniti Analyzer. The post PCR steps are completely automated. 24 samples can be tested in one run. It takes 4.5 hrs for the first sample to be processed and scanned and one chip will be processed every 30 minutes thereafter.

For more information about ICON Central Laboratories Research & Development services, please email carol.rosenthal@iconplc.com.



A Symbol of Excellence

Pharmacogenomics

Genetic Testing for CYP450 Enzymes

Authored by Alamelu Chandrasekaran, PhD

The pharmaceutical industry is slowly moving away from the traditional model. New drugs are being designed as target specific and to treat specific patient populations. The system of ‘one size fits all’ will eventually be replaced by the new trend of personalized medicine. Pharmacogenomics, the science of how genomic variations in people alter the response to drugs, will facilitate the development of personalized medicine.

Even though the term pharmacogenomics is relatively new, it evolved from the field of pharmacogenetics which has existed since 1952. It has been well known for decades that genetic components have a major impact on drug metabolism, toxicity, absorption and distribution. Human genome and HapMap projects have revealed a wealth of information on genetic variations in the human genome. Advanced high throughput techniques enable the identification of multiple markers associated with diseases and drug responses.

Pharmacogenomics involves the study of the genetic basis for individual differences in response to drugs by investigating individual drug metabolism and its relationship with genetic variants. Genetic variations can manifest as differences in the drug targets or as differences in the enzymes that metabolize drugs. While differences in the drug target can influence how the drug works, differences in metabolizing enzymes can lead to variations in efficacy and toxicity. Testing specific genetic variations can be used to predict whether a patient will have a good response, poor response or no response to a drug.

Pharmacogenomic strategies have been used in successful drug development in the field of cancer. Stratification of patients based on target-specific genetic markers has been shown to be useful to increase the drug response rate. Patient stratification based on tumor genetics such as the HER2 gene or protein expression for Herceptin treatment has been shown to increase response rates to Herceptin. Only patients who express this gene will respond to treatment with Herceptin.

Mutations in the KRAS gene can affect response to EGFR inhibitors Erbitux (Cetuximab) and Vectibix (Panitumumab) drugs that are used to treat metastatic colon cancer. In patients with non-mutated wild type KRAS tumors, Vectibix significantly increased progression-free survival. Metastatic colorectal cancer patients who carry the wildtype version of the KRAS gene are much more likely to benefit from the monoclonal antibody Erbitux than patients with the mutated form of the gene (1,2).

On the other hand, pharmacogenomic applications of cytochromeP450 (CYP450) enzymes, a major class of drug metabolizing enzymes, are increasing as the majority of the drugs are substrates of one of the CYP enzymes. Genetic polymorphisms within P450 enzymes lead to loss of enzyme activity and affect the pharmacokinetics of drugs, mainly the metabolism. The differences in drug metabolism lead to differential pharmacological effects which reflect on safety (toxicity) and efficacy of drugs.

CytochromeP450 System

CytochromeP450 enzymes (CYP450) belong to one of the largest families of enzyme proteins involved in oxidative drug metabolism. CYP450 enzymes are heme containing proteins that use iron to oxidize drugs and other xenobiotics to dispose of potentially harmful substances by making them water-soluble. Metabolic clearance is the major function of CYPs. They are responsible for the metabolism of numerous drugs that are available in the market. CYP450 enzymes are predominantly expressed in the liver, the major organ involved in detoxication, but a remarkable amount can also be found in the small intestine. CYP families contain genes that have at least 40% sequence homology. Humans have 18 families of cytochrome P450 genes. There are 43 subfamilies containing members with at least 55% identity. There are about 57 genes coding for the various cytochrome P450 enzymes in man. Some of the important CYP450 enzymes are CYP3A4, CYP3A5, CYP2C9, CYP2C19 and CYP2D6. The importance of CYPs is well acknowledged as only six of the CYPs metabolize almost 90% of the drugs.

Genetic variations in the CYP450 enzymes have profound implications on drug response and adverse drug reaction as they alter the metabolism of the drugs (substrates) that are being metabolized by them. Mutations in CYP genes can result in less active or inactive forms of CYP enzymes that are unable to breakdown and efficiently eliminate drugs from the body (slow metabolism) and can cause adverse drug reactions or toxicity in patients. Multiple copies of the wild type gene can result in ultra rapid clearance of drugs. The bimodal distribution of poor and extensive metabolizers and the relationship between metabolizer status and genetic inheritance with respect to CYP2D6 was studied as early as 1977 (3).

CYP2D6 metabolizes about 30% of all prescribed drugs. Approximately 7-10% of Caucasians are poor metabolizers of drugs metabolized by CYP2D6 in whom the enzyme is not expressed due to mutations. Approximately 1% of Caucasians are ultra rapid metabolizers carrying multiple copies of the gene. The CYP2D6 gene contains about 80 mutations mainly in the form of single nucleotide polymorphisms (SNPs). CYP2D6*3, *4 and *5 together can predict 90% of poor metabolizers. Some of the drugs cleared by CYP2D6 are antidepressants, antiarrhythmics, analgesics, antipsychotics, opiates and tamoxifen.

One of the isoforms of cytochromeP450, CYP2C9, metabolizes the anticoagulant drug warfarin which has a very narrow therapeutic range. There is a large inter-individual variability in dose requirement for this medicine. CYP2C9 was the first gene identified to influence this variation in requirement. Individuals with genetic variations in this enzyme are slow metabolizers of warfarin and are at an increased risk of bleeding when treated with standard doses of the drug (4,5) thus necessitating CYP2C9 genotyping in these individuals prior to treatment. Mutations in VKORC1 (vitamin K epoxide reductase) have also been found to impact warfarin metabolism. The FDA estimates that 2 million people take warfarin every year in the United States to prevent blood clotting, heart attacks and stroke.

A recent paper published in the journal *Blood* has evaluated 29 genes and 183 polymorphisms in 1496 patients (6) to study their relationship with warfarin dose requirements. The study has clearly indicated that pharmacogenetic testing of CYP2C9 and VKORC1 (vitamin K epoxide reductase) is necessary to improve the clinical outcome in patients who need warfarin therapy. Genetic testing can also be used to fix dosage according to the metabolizer status or even to change the drug in poor responders. About 1-2% of Caucasians carry poor metabolizer genotypes. A large number of drugs, including the anti-inflammatory drugs celecoxib, diclofenac and ibuprofen, are substrates of CYP2C9.

CYP2C19 metabolizes 15% of prescribed drugs. 3-6% of Caucasians and 15-20% of Asians have inactive form of this enzyme. Proton pump inhibitors (Prilosec), tricyclic antidepressants, selective serotonin reuptake inhibitors and antiepileptics are some important substrates of this enzyme. CYP2C19*2 and the CYP2C19*3 mutations account for approximately 90 % of the poor metabolizers.

Clopidogrel, an antiplatelet drug recommended for coronary heart conditions, needs to be activated by CYP2C19. A large study involving 2,208 patients with myocardial infarction receiving clopidogrel has reported that patients carrying CYP2C19 loss of function alleles were associated with increased risk of death, subsequent cardiovascular events and stroke (7,8)

CYP3A4 is the most prevalent CYP in the body involved in the clearance of almost 55% of the drugs including calcium channel blockers, benzodiazepines and HIV protease inhibitors. About 5-10% of Caucasians have inactive form of this enzyme.

Adverse Drug Reactions

Adverse drug reactions (ADRs) are one of the leading causes of death in United States. A study of hospitalized patients published in the United States reported that, in 1994, adverse drug reactions accounted for more than 2.2 million serious cases and over 100,000 deaths, making adverse drug reactions (ADRs) one of the leading causes of hospitalization and death in the United States(9). Pharmacogenomic testing may offer the best solution to this

INFINITI™ Assays

INFINITI™ Assays

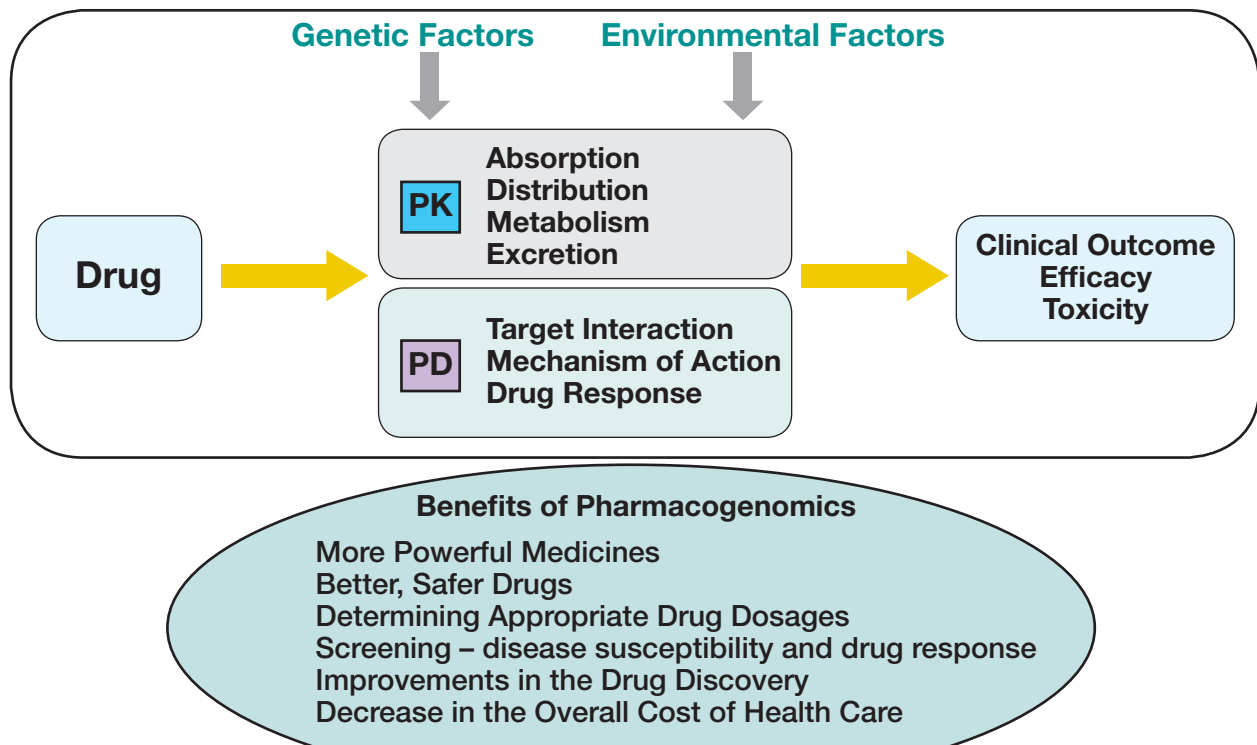
Factor II (IVD)	CHEK-2*
Factor II Plus*	EGFR (mutations)*
Factor V (IVD)	5-FU*
MTHFR*	MDR-TB*
FII-FV Panel (IVD)	Respiratory Viral Panel*
FII-FV-MTHFR Panel*	STD-6 Panel*
CFTR31*	STD-6 Quad*
Ashkenazi Jewish Panel*	CT-NG Quad*
Warfarin Assay (IVD)	NTM (Nontuberculous
HPV Genotyping*	Mycobacteria)*
HPV Quad*	
NAT-2*	

INFINITI™ CYP450 Assays

CYP2C9/VKORC1*	- 14 analytes
CYP2D6*	- 16 analytes
CYP 2D6T *	- 8 analytes
CYP2C19	- 9 analytes
CYP3A4*	- 5 analytes
CYP3A5*	- 8 analytes
MDR-1*	- 6 analytes
UGT1A1*	- 4 analytes

Custom assays can be developed.

Pharmacogenomics – Variation in Drug Response



CYP450 Testing ... continued :

problem as mutations can predict toxic responses. The studies mentioned above on 2C9 are large scale studies that have proven the importance of 2C9 genotyping to avoid an ADR during warfarin therapy. There are several other drugs that require pharmacogenetic tests for toxic response. For example, reduced expression of Uridine glucuronosyltransferase 1A1 (UGT1A1), due to polymorphisms within the gene, is associated with increased incidence of toxicity in patients receiving the drug irinotecan for colon cancer treatment. Patients with HLA-B57 allele have been shown to have a serious and sometimes fatal hypersensitivity reaction to abacavir therapy.

Drug Interactions

CYP450 enzymes can be inhibited or induced by certain other drugs. The amount of inhibition depends on the dosage of inhibitory drug. Inducers of CYPs increase the enzyme synthesis, leading to a decrease in the concentration of the drug that is metabolized by CYPs. Interaction between the two drugs may result in different consequences in different genotypes of the enzyme. An extensive metabolizer can become a poor metabolizer by using a drug that is an inhibitor of the enzyme. Inducers or inhibitors cannot change the genetically poor responder as the enzyme activity is genetically determined to be low. Proton pump inhibitors reduce the activity of clopidogrel, an anti-platelet agent by inhibiting CYP2C19 activity. Rifampicin, and anticonvulsants like carbamazepine and phenytoin, are inducers of CYP3A4. Rifampicin is an inducer of 2C9 also. Co-medication of rifampicin with CYP2C9 substrate will decrease the drug's concentration due to an increase in 2C9 activity. Based on the metabolizer status, dose adjustments or substitutions may be considered when inhibitors or inducers of CYPs are prescribed.

CYP450 Genetic Polymorphism Assays

Following the pharmacogenetic labeling on warfarin, several diagnostic companies have developed CYP2C9/VKORC1 genotyping assays. Assays to test CYP2C19 and 2D6 are also available. They








are either real time PCR assays or array-based assays containing a panel of genetic variants. The AmpliChip CYP450 test (Roche) is the first FDA-cleared pharmacogenetic test for analysis of the CYP2D6 and CYP2C19 genes. It is a microarray test developed by Affymetrix. Autogenomics, a California based company, has developed microarrays for all CYP enzymes. The microchips contain a panel of important genetic variants of these enzymes. Luminex has developed bead based liquid arrays for CYP2C9, 2C19 and 2D6 panels. Osmetec has developed a microarray based IVD test (eSensor) for warfarin sensitivity.

Conclusion

The benefits of pharmacogenomics include safe and effective drugs with no adverse drug reactions. Incorporation of genetic tests into clinical trials is necessary to develop effective new drugs. Selection of responder populations, based on genomic testing prior to the start of clinical trials will increase the efficacy of drugs and reduce the cost of trials.

Drugs labeled by the FDA for pharmacogenomic testing are increasing. Numerous new drugs are expected to obtain genetic labeling. The FDA's Critical Path Initiative supports novel drug development strategies to improve the quality of healthcare. This also includes identification biomarkers that can predict the safety and effectiveness of drugs. It is expected that submission of pharmacogenomic data with new drugs will become mandatory in the near future. Pharmacogenomics will be important along the entire drug discovery and development process, from target discovery to clinical trials, to develop new drugs that are safe and effective. Pharmacogenomic tests may revolutionize modern medicine to provide the patients with tailored therapy based on their genetic make-up. Individualized treatment strategies will reduce the risk of side effects and provide cost effective healthcare. Pharmacogenomics will play a major role in future medicine, diagnostic and drug industry.

SAMPLE PROCESS FLOW

INFINITI™ WORKFLOW		Time	
Extraction from blood samples:  Use Manual Extraction OR Automatic Extraction		Manual 90 Minutes	Auto 30 Minutes
Amplification from extracted samples: Start with 25ng/uL of extracted DNA  Amplification mix + Tag Enzyme + DNA → Thermocycler		90 Minutes	
Primer Extension of PCR samples: Primer with Anti-ZipCode Technology  Primer Extension Mix + Amplified Samples → Anti-ZipCode + Amplicon → Detection Primer + Fluorescent nucleotides		90 Minutes	
Hybridization to the BioFilmChip Microarrays: ZipCode/Anti-ZipCode Technology  Hybridization Chip + Wash Buffer		90 Minutes	
Washing the BioFilmChip Microarrays: Removing unincorporated dyes  Hybridization Chip + Wash Buffer		25 Minutes	
Detection / Analysis / Result Report: With built in confocal microscope  INFINITI Software calculations		5 Minutes	
Total Time To First Result: 		Manual 390 Minutes	Auto 330 Minutes

AUTOMATED

CYP450 Testing ... continued from page 3

References:

1. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R et al.: Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol.* 2008;26:1626-1634.
2. Lievre A, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, Ychou M, Bouche O, Landi B, Louvet C et al.: KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol.* 2008;26:374-379.
3. Mahgoub A, Idle JR, Dring LG et al. Polymorphic hydroxylation of Debrisoquine in man. *Lancet.* 1977;2(8038):584-586.
4. Wadelius M, Sörlin K, Wallerman O, Karlsson J, Yue QY, Magnusson PK, Wadelius C, Melhus H. Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. *Pharmacogenomics J.* 2004;4(1):40-8.
5. Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padriani R. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. *Clin Pharmacol Ther* 2002;72:702-710.
6. Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghorri MJ, Bumpstead S, Holm L, McGinnis R, Rane A, Deloukas P. The largest prospective warfarin-treated cohort supports genetic forecasting. *Blood.* 2009;113(4):784-92
7. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, Steg PG, Ferrières J, Danchin N, Becquemont L; French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med.* 2009;360(4):363-75
8. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, Sabatine MS. Cytochrome P-450 polymorphisms and response to clopidogrel. *N Engl J Med.* 2009;360(4):354-62.
9. Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA.* 1998;279(15):1200-1205.

In the Next Issue of R & D Solutions

Look for our next issue of R&D Solutions which will include information on the Luminex Platform.



LifeMatch Fluoroanalyzer
Powered by xMAP™ technology from Luminex

Future Areas for Expansion

The R&D team at ICL focuses on Cellular Immunology and Molecular Diagnostics as two areas of expansion in Research and Development. These two sciences have seen the greatest rate of growth over the past five years. In tandem with this, plans are underway to follow the industry trend to develop new Biomarker assays in all areas of clinical diagnostics.

To learn more, please contact Carol Rosenthal:

Tel: 1 (631)-306-5478
Email: carol.rosenthal@iconplc.com



A Symbol of Excellence

Central Laboratories