Reliable Genotyping with IVD Cleared Assays

xTAG® CYP2D6 Kit v3 and xTAG® CYP2C19 Kit v3 assays may aid in determining therapeutic strategies for drugs metabolized by CYP2D6 and CYP2C19 gene products.

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Introduction

Physicians have long been aware of the subtle differences between patients and their responses to medications. The recognition that a part of this variation is inherited, and therefore predictable, created the field of pharmacogenetics several years ago.

Pharmacogenetics studies the influence of genetic variation on drug response. Genetic variation is considered an important source of variability in drug response and contributes to 25% - 50% of inappropriate drug responses.[1] It has the potential to negatively impact effectiveness of drug therapy ('drug efficacy') and increases the risk for dangerous side effects, termed adverse drug reactions (ADRs). Linking the genetic source of variability to drug response is often clinically significant and meaningful.[2, 3]

A patient's response to a drug is often linked to common genetic variations present in their genes. One type of genetic variation is the single nucleotide polymorphisms (SNPs). Knowing the types of SNPs genetic variations present in a patient can help predict the associated drug response. This can not only help physicians individualize drug therapy, it will also help improve effectiveness of the drug, decrease the chance of adverse drug reactions and reduce healthcare costs.[4]

Pharmacogenetics 101

(Reference: http://www.genome.gov)

- Single Nucleotide Polymorphism (SNP)—Genetic variation arising from substitution of one base pair in DNA for another base pair is referred to as a SNP. SNP is a genetic mutation in the DNA that can result in a disease phenotype.
- Haplotype—Combinations of several SNPs together on the same chromosome.
- Alleles—Alternative forms of a gene that arise by mutations in the DNA.
- Genotype—An individual's collection of genes. The term also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the gene is used to make protein.
- Phenotype—The expression of the genotype contributes to the individual's observable traits.

Accurate prediction about drug response is crucial for individualized treatment. This is best made by combining an individual's genetic data with clinical findings and classifying patients into subpopulations based on their response to a specific drug.[5] Using this approach, health care providers can move beyond the "one-size-fits-all" strategy and identify treatments that are more personalized.

The discovery of genetic factors such as the cytochrome P450 (CYP) drug metabolizing genes and several years of subsequent clinical research have added to the understanding of the clinically relevant genetic variations that may help predict drug response.

Genetic Variation and Drug Efficacy

The extent to which patients metabolize drugs has a significant impact on the effectiveness of their therapeutic effect.

Genetic variation in CYP450 metabolizing genes plays a major role in variability in drug response.[7] To a large extent the CYP450 genotype of a patient determines the level of enzyme activity ('phenotype') which can be classified into four groups:

- **Extensive metabolizers (EMs)** have normal enzymatic activity, and carry either two wild-type alleles, or one wild-type allele and one decreased activity or null allele.

- **Intermediate metabolizers (IMs)** have decreased enzymatic activity, and carry either two decreased activity alleles, or one decreased activity allele and one null allele.
- **Poor metabolizers (PMs)** have absent enzymatic activity, and carry two null alleles.
- **Ultra-rapid metabolizers (UMs)** have increased enzyme activity, and have gene duplications or multiplications of the CYP2D6 gene (more than two copies of the gene)[8]

Typical drug efficacy rates range from 25% to 80%, with most drugs falling in the range of 50 to 60%.[5] For example, only 50-60% of patients experience improved outcome with drug therapy used for depression, schizophrenia and cardiac arrhythmias (Table 1).[8]

Table 1: Drug Efficacy Rates For Major Drugs in Selected Therapeutic Areas

Therapeutic Area	Efficacy rate (%)
Cardiac arrhythmias	60
Schizophrenia	60
Depression (SSRI)	62
Analgesics (Cox-2)	80

Genetic Variation and Adverse Drug Reactions (ADR)

Adverse drug events due to variability in drug responses are often preventable[9] and remain an underappreciated clinical issue. The Food & Drug Association Adverse Events Reporting System (FAERS) estimated 800,000 ADRs in the U.S. and Europe combined for the year 2011.[10] The incidence of serious & fatal ADRs has been rising with the increase in the number of medications prescribed. An estimated $3.5 billion is spent on additional medical cost associated with ADRs annually and at least 40% of this may be preventable.[11]

Cytochrome P450 2D6 (CYP2D6) Enzyme

Drugs may be metabolized by more than one pathway involving several enzymes of the cytochrome P450 class. Cytochrome P450 enzyme 2D6 (CYP2D6) alone is thought to be active in the enzymatic breakdown of 20-25% of all medicines prescribed[12] including antidepressants, antipsychotics, opioids, beta-blockers, antiarrhythmics, and the drug tamoxifen.

Genetics of CYP2D6

Most individuals have two CYP2D6 alleles, one inherited from each parent. The combination of these two alleles ('genotype') determines the overall level of CYP2D6 enzyme activity, or phenotype, particular to that combination.

Cytochrome P450 2C19 (CYP2C19) Enzyme

Cytochrome P450 enzyme 2C19 (CYP2C19) metabolizes many clinically important drugs including proton pump inhibitors, antidepressants, the antiplatelet drug clopidogrel, and the antifungal voriconazole.[13]

Genetics of CYP2C19

Like CYP2D6, most individuals are born with two CYP2C19 alleles The combination of these two alleles determines the overall level of CYP2C19 enzyme activity, or phenotype, particular to that combination.

The mutations in the CYP2C19 gene are heritable. Up to 34 different variations in the gene sequence have been described for CYP2C19.[14] The CYP2C19*1 allele is considered the wild-type, or "normal" allele, with "normal" enzyme activity.

Table 2: Prevalence of CYP2C19 Phenotypes in the General Population[15]

2C19 Phenotype	Percent of patients with phenotype
Extensive	35-50
Intermediate	18-45
Poor	2-15
Ultra-rapid	5-30

Conclusion

Laboratory techniques to detect drug response variability exist currently. Phenotyping and /or genotyping are primary methods used. Phenotyping is carried out by measuring enzyme activity directly using a probe drug whose metabolism is known to be solely dependent on the particular CYP enzyme. However, using a probe drug to measure individual phenotypes has limitations. Measuring concentration at various time points requires collecting multiple specimens at fixed times (typically at 8 hours post-administration). The individual is also exposed to possible unfavorable side effect of the probe-drug. Additionally, the metabolism of the probe drug may be affected by interfering drugs, disease status and other environmental factors (such as a patients overall health, weight, age, diet).

The drug-metabolizing phenotype of an individual can also be predicted using assays that determine genotype from a patient sample. Genotyping results are not affected by drugs, diet or environmental factors. Genotyping assays by molecular methods are fast, reliable and accurate. The interpretation of the genotype result to the phenotype is based mainly on literature, and on the physician's judgment.

Identification of patient genotypes for clinically relevant CYP genes can help physicians tailor drug treatment to patients through the selection of appropriate therapies. These measures may improve a physician's ability to impact patient outcome by ensuring maximum drug efficacy with minimal adverse drug reactions.[6]

xTAG CYP2C19 Kit v3 (EU-IVD) Intended Use

The xTAG CYP2C19 Kit v3 (EU-IVD) is an in vitro diagnostic test used to simultaneously detect and identify a panel of nucleotide variants found within the highly polymorphic CYP450 2C19 gene, located on chromosome 10q24, from genomic DNA extracted from EDTA or citrate anticoagulated whole blood samples.

The xTAG CYP2C19 Kit v3 is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for the therapeutics that are metabolized by the CYP2C19 gene product, specifically *1, *2, *3, *4, *5, *6, *7, *8, *9, *10, and *17.

This kit is not indicated for stand-alone diagnostic purposes. The information provided from this test may supplement decision making and should only be used in conjunction with routine monitoring by a physician. Because of the variability in the knowledge of clinical utility with specific drugs that are metabolized by CYP2C19, clinicians should use professional judgment in the interpretation of results from this test. Results from this type of assay should not be used in predicting a patient's response to drugs for which the drug metabolizing enzyme activity of that allele, or the drug metabolic pathway, has not been clearly established.

xTAG CYP2D6 Kit v3 (EU-IVD) Intended Use

xTAG® CYP2D6 Kit v3 is a device used to simultaneously detect and identify a panel of nucleotide variants found within the highly polymorphic CYP2D6 gene located on chromosome 22 from genomic DNA extracted from EDTA and citrate anticoagulated whole blood samples. This kit can also identify gene rearrangements associated with the deletion (*5) and duplication genotypes. xTAG CYP2D6 Kit v3 is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for therapeutics that are metabolized by the CYP2D6 gene product. This kit is not indicated for stand-alone diagnostic purposes. This test is not intended to be used to predict drug response or non-response.

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