Technical Brief

Genetic Test of Cytochrome P450 2C19 (CYP2C19) for Drug Metabolism

Background

Cytochrome P450 (CYP) enzymes, largely present in the liver, regulate the metabolism of several prescription drugs. CYP2C19 is an isoenzyme that mediates the metabolic activation and elimination, and hence the therapeutic effect of a variety of drugs including antiplatelet agents, anticonvulsants, anti-depressants, antineoplastic drugs, antiretroviral/antifungal drugs, antimalarial drugs, antithrombotics, antiulcer drugs, beta blockers and proton pump inhibitors. Drugs metabolized through CYP2C19 are listed in Table 1.

The ability to metabolize drugs that are CYP2C19 substrates differs among patients resulting in wide variability in their response to specific drug therapy. A major cause of such inter-individual variability is associated with variations in *CYP2C19* gene encoding CYP2C19 enzyme. Prevalence of a specific genetic variant is dependent on racial and ethnic background. Over 25 variants have been identified for the *CYP2C19* gene, and about a dozen variants, which are functionally impaired, can lead to the production of CYP2C19 with high, low or no enzyme activity causing abnormal levels of drugs and their derivatives. This can result in adverse events including toxicity, sub-therapeutic effect or non-responsiveness.

On the basis of CYP2C19 metabolic ability, individuals can be broadly classified into four phenotypes: normal (extensive), intermediate, poor and ultra metabolizers. Since the therapeu-tic effect of a specific drug is dependant on whether it is metabolically activated or cleared/inactivated by CYP2C19, determining the phenotype of patients from their genotype may help to manage individualized therapy and limit adverse events.

Clinical Significance of Genetic Testing

The CYP2C19 isoenzyme metabolizes about 5-10% of all prescription drugs, and poor metabolizers taking those drugs may be predisposed to adverse events due to underdosing or overdosing, depending on whether it is a pro-drug or active drug, respectively. It is estimated that about 2-14% of the U.S. population are poor metabolizers. Pharmacokinetics of drugs and consequent therapeutic efficacy depend on the metabolic rate of activation or elimination. Poor metabolizers are likely to have markedly reduced or no conversion rate of pro-drugs (examples: clopidogrel; tamoxifen) to active drugs resulting in either sub-therapeutic effect or non-responsiveness (underdosing), which eventually require alternative treatment or increased dosage. Poor metabolizers may also have a reduced capacity to eliminate active drugs (examples: Prilosec, generic name – omeprazole; Valium, generic name – diazepam) leading to accumulation and resulting in toxicity and life-threatening side effects (overdosing).

Such adverse effects can be predicted by determining the genotype-phenotype associations (Figure 1). The homozygous wild type allele of *CYP2C19*, designated *CYP2C19*1*, is defined as an extensive metabolizer (EM). Nine common variants of *CYP2C19* gene associated with impaired drug metabolism include *2 to *10. While the wild-type form of the *CYP2C19* gene (*1) encodes an enzyme with normal functional activity, genetic variants (*2 to *8) encode enzymes with non-functional activity and two other variants (*9 and *10) have decreased functional activity. The most common poor metabolizer (PM) phenotypes are from *CYP2C19* with homozygous *2 and *3 alleles. Homozygous *4 to *8 alleles are less common PM. One of the variants (*17) confers augmented enzyme activity, leading to an ultra metabolizer (UM) phenotype. Inheritance of one normal allele and one non-function allele can result in intermediate metabolizer (IM) phenotype.

This genetic test is used to identify patients at risk for adverse events due to impaired metabolism by:

Detecting 6 variants of *CYP2C19* gene responsible for non-functional metabolic activity:

- *CYPC2C19*2, *3, *4, *6, *7*, and *8*

Detecting 2 variants of *CYP2C19* gene responsible for decreased functional metabolic activity:

- *CYPC2C19*9* and *10*

Detecting one variant of *CYP2C19* gene responsible for increased functional metabolic activity:

- *CYPC2C19*17*

Interpretation

Interpretation of the assay is based on the presence of specific genetic variants of *CYP2C19* (Table 2).

1. Individuals with *CYP2C19* variant alleles designated *2, *3, *4, *6, *7 and *8 have non-functional activity compared to *1 (wild-type) individuals. Administration of pro-drug such as clopidogrel can result in a markedly reduced or no conversion to their active metabolite, and sub-therapeutic effect or non-responsiveness, which require alternative treatment or increased dosage. Conversely, administration of active drugs, such as diazepam and omeprazole, may result in impaired elimination, and potential risk of drug toxicity and life-threatening side effect, which requires alternative treatment or reduced dosage.

2. Individuals with *CYP2C19* variant alleles designated *9 and *10 have decreased functional activity. Such individuals may have sub-therapeutic effect for pro-drugs. Conversely, administration of active drugs may result in impaired elimination, and potential risk of drug toxicity.

3. Individuals with *CYP2C19* variant alleles designated *17 have augmented enzyme activity compared to *1 (wildtype) individuals, leading to increased conversion of pro-drugs to active metabolites with potential risk for toxicity requiring reduced dosage. Conversely, it may also lead to rapid elimination of active drugs and sub-therapeutic effect.

4. Individuals with a *CYP2C19* genotype comprised of one of the non-function alleles (*2, *3, *4, *6, *7 or *8) and *17 have an unknown metabolizer phenotype. Due to the low population frequency of these alleles, the level of CYP2C19 activity cannot be predicted based on genotype.

5. Individuals with a *CYP2C19* genotype comprised of one of the reduced function alleles (*9 or *10) and *17 have an unknown metabolizer phenotype. Due to the low population frequency of these alleles, the level of CYP2C19 activity cannot be predicted based on genotype.

6. Prevalence of *CYP2C19* gene variants differs depending on ethnic background. The frequency of allele *CYP2C19*2* has been reported as approximately 30-35% of Asians, and 15-26% of Caucasian and African Americans. The frequency of allele *CYP2C19*3* has been reported as approximately 10% of Asians, and less than 2% of Caucasian and African Americans.

7. Genotype-phenotype interpretation should be made in the context of a patient's clinical condition and concomitant medications, which may be substrates, inhibitors and inducers of CYP2C19.

Methodology

An array-based test kit employing Infiniti analyzer (Auto-Genomics Inc., CA) is used for genotyping CYP2C19 variants. The assay involves a multiplex PCR amplification of genomic DNA followed by allele-specific primer extension using fluorescently labeled dCTP and hybridization on to a microarray
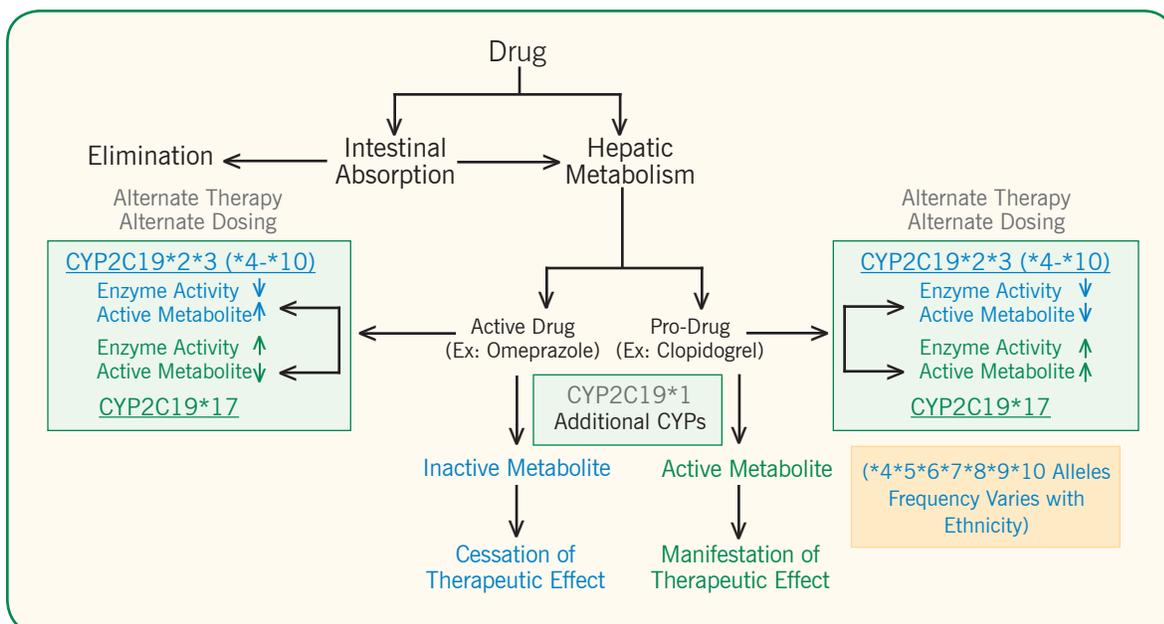

FIGURE 1. CYP2C19 MEDIATED DRUG METABOLISM

coated with capturing oligonucleotides, which are specific for complementary oligonucleotides linked to the allele specific primer-extended products.

A built-in confocal microscope is enabled to capture fluorescent signal from the pre-determined hybridization spots corresponding to specific products and genotypes deciphered from signal ratio.

Limitations of the Assay

Analysis for specific genetic variants detected in this test does not rule out the possibility of the presence of other variant alleles in CYP2C19 or other genes that may influence drug effect and metabolism. CYP2C19 variant alleles are important in the metabolism of drugs including but not limited to antithrombotics, anticonvulsants, antidepressants, antiulcer and antimalarial drugs, and proton pump inhibitors. A *2 or *3 result for CYP2C19 is associated with a poor metabolizer phenotype for all drugs metabolized by CYP2C19. Non-genetic factors such as concurrent medications, impaired hepatic function, obesity, insulin resistance and non-compliance can also affect CYP2C19 metabolism. These can lead to an increase or decrease in function relative to the predicted genotype. Co-administration of drugs metabolized by CYP2C19 may increase or decrease the CYP2C19 activity. CYP2C19 genotyping should not replace clinical monitoring of patients, when required.

TABLE 1. SUMMARY OF DRUGS METABOLIZED THROUGH CYTOCHROME P450 2C19

1. Substrates metabolized through cytochrome P450 2C19:
    Antiplatelet agents: clopidogrel
    Anticonvulsants: mephenytoin, phenytoin, primidone, mephenytoin,
    Antidepressants: amitriptyline, citalopram, S-citalopram, clomipramine, imipramine, fluoxetine, escitalopram, moclobemide, trimipramine, sertraline
    Antineoplastic drugs: cyclophosphamide
    Antiretroviral/antifungal drugs: nelfinavir, voriconazole, vfend
    Proton pump inhibitors: lansoprazole, omeprazole, pantoprazole
    Miscellaneous drugs: diazepam, progesterone, propranolol, R-warfarin, proguanil, malarone, carisoprodol, flunitrzaepam, soma

2. Inhibitors of cytochrome P450 2C19: Co-administration of the following drugs will decrease the rate of metabolism of drugs and increase the possibility of toxicity.
    Chloramphenicol, cimetidine, delavirdine, efavirenz, felbamate, fluconazole, fluoxetine fluvoxamine, indomethacin, isoniazid, ketoconazole, lansoprazole, modafinil, omeprazole, oral contraceptives, oxacarbazepine, probenicid, ticlopidine, topiramate, voriconazole

3. Inducers of cytochrome P450 2C19: Co-administration of the following drugs will increase the rate of excretion of drugs and reduce the drug's effectiveness.
    Carbamazepine, ginko biloba, prednisone, rifampin, secobarbital, St. John's wort

TABLE 2. CYTOCHROME P450 2C19 GENOTYPE AND PHENOTYPE FOR DRUG METABOLISM

Genotype	Allele	Phenotype / Enzyme Activity
CYP2C19*1	wild type	EM / Normal function
CYP2C19*2	681 G>A	PM / Non-function
CYP2C19*3	636 G>A	PM / Non-function
CYP2C19*4	1 A>G	PM / Non-function
CYP2C19*6	395 G>A	PM / Non-function
CYP2C19*7	IVS 5+2T>A	PM / Non-function
CYP2C19*8	358 T>C	PM / Non-function
CYP2C19*9	431 G>A	IM / Decreased function
CYP2C19*10	680 C>T	IM / Decreased function
CYP2C19*17	-806 C>T	UM / Increased function

Abbreviations: EM (Extensive Metabolizer); IM (Intermediate Metabolizer); PM (Poor Metabolizer); UM (Ultra Metabolizer)

References

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Test Overview

Test Name	Genetic Test of Cytochrome P450 2C19 (CYP2C19) for Drug Metabolism
Methodology	Multiplex PCR and array hybridization assay
Reference Range	An interpretive report will be provided.
Specimen Requirements	Testing Volume/Size: 3 mL; Type: Whole blood; Tube/Container: EDTA (Lavender); Collection Temperature: Refrigerated
Ordering Mnemonic	2C19PL
Billing Code	88307
CPT Codes	83891, 83900, 83901 x4, 83914 x18, 83912

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