Genetic Test of Cytochrome P450 2C19 (CYP2C19) for Clopidogrel Therapy

Background Information

Clopidogrel is an oral antiplatelet drug used to treat acute coronary syndromes (ACS) including patients undergoing percutaneous coronary intervention (PCI), myocardial infarction (MI), cerebrovascular disease and peripheral arterial disease. Clopidogrel is typically prescribed at a daily dosage of 75 mg with or without an initial loading dose of 300-600 mg, as needed. In combination with a daily dose of 75-325 mg aspirin, it is used at 75 mg dosage to prevent stent-thrombosis following PCI.

Clopidogrel administered as a pro-drug is largely (~85%) hydrolyzed by esterases into inactive metabolites, and only approximately 15% is converted into an active form by hepatic Cytochrome P450 (CYP) enzymes, primarily by isoenzyme CYP2C19. The active metabolite binds irreversibly to the platelet adenosine diphosphate (ADP) receptor P2RY12 to inhibit platelet aggregation and consequent thrombosis (Figure 1). The antithrombotic effect of clopidogrel is not optimal in all patients, and up to 30% do not benefit from the therapy as determined from the measurement of residual platelet reactivity. Non-response or sub-optimal response to therapy can stem from pharmacokinetics and pharmaco-dynamics, co-administration of other drugs and predisposition due to genetic, clinical and cellular factors.

Among the CYPs, certain variations in the gene encoding CYP2C19 enzyme have been associated with poor metabolism of clopidogrel, and subsequent decreased formation of active metabolite. The Food and Drug Administration (FDA) has issued a black box warning about the reduced effectiveness of clopidogrel in patients who are poor metabolizers of the drug. The FDA has indicated the availability of tests to identify genetic differences in CYP2C19 function, and advised healthcare professionals to consider alternate therapy or dosing strategies for poor metabolizers.

Clinical Significance of Genetic Testing

Clopidogrel is converted into an active metabolite in the liver by several CYP enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and CYP3A5 (Figure 1). Of these, CYP2C19 plays a major role in the generation of active metabolite. There is a wide variability observed among patients in response to clopidogrel therapy that is attributed to at least three common variants in *CYP2C19* gene. The wild-type form of the *CYP2C19* gene (*1) encodes an enzyme with normal activity (extensive metabolizer) that current drug dosing recommendations are based on. Two variants (*2 and *3) encode enzymes with non-functional activity (poor metabolizer). In contrast, a third variant (*17) encodes an enzyme with increased activity (ultra metabolizer).

As a result, *CYP2C19*2* and *CYP2C19*3* variants confer non-functional or markedly reduced metabolic status and have been associated with non-responsiveness to clopidogrel reflected in reduced platelet inhibition and poor outcomes including increased risk for stent thrombosis, MI, stroke and death. On the other hand, *CYP2C19*17* is associated with an increased response to clopidogrel and risk for bleeding. Accordingly, while sub-optimal response to clopidogrel can lead to thrombosis, super-optimal response can result in bleeding. Thus, the genetic testing of patients to determine *CYP2C19* gene variants can enable individualized antithrombotic therapy. However, this genotyping assay should be used in conjunction with the aspirin/clopidogrel aggregation assay to determine platelet response to clopidogrel.

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

Detecting two variants of *CYP2C19* gene responsible for non-functional activity.

- *CYP2C19*2* and *CYP2C19*3*

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

- *CYP2C19*17*

Interpretation

Interpretation of the assay is based on the presence of specific genetic variants of *CYP2C19*. The genotypes including alleles and phenotypes of *CYP2C19* are shown in Table 1.

1. Individuals with *CYP2C19* variant alleles designated *2 and *3 have non-functional enzyme activity compared to *1 (wild-type) individuals, leading to absent or markedly decreased metabolite that may require increased maintenance doses of clopidogrel.
2. Individuals with *CYP2C19* variant alleles designated *17 have increased enzyme activity compared to *1 (wildtype) individuals, leading to increased levels of active metabolite and may require lower maintenance doses of clopidogrel.
3. Individuals with a *CYP2C19* genotype comprised of either *2 or *3 and *17, and allele with combination of *4,*6-*10 or *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.

TABLE 1. CYTOCHROME P450 2C19 GENOTYPE AND PHENOTYPE FOR CLOPIDOGREL

Genotype	Allele	Phenotype/Enzyme Activity
*CYP2C19 *1/*1*	wild type/wild type	EM / Normal function
*CYP2C19 *1/*2*	wild type/681 G>A	IM / Decreased function
*CYP2C19 *1/*3*	wild type/636 G>A	IM / Decreased function
*CYP2C19 *2/*2*	681 G>A/681 G>A	PM / Non-function
*CYP2C19 *2/*3*	681 G>A/636 G>A	PM / Non-function
*CYP2C19 *3/*3*	636 G>A/636 G>A	PM / Non-function
*CYP2C19*1/*17*	wild type/-806 C>T	UM / Increased function
*CYP2C19*17/*17*	-806 C>T/-806 C>T	UM / Increased function
*CYP2C19*2 or *3/*17*	681 G>A or 636 G>A/-806 C>T	Unknown

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

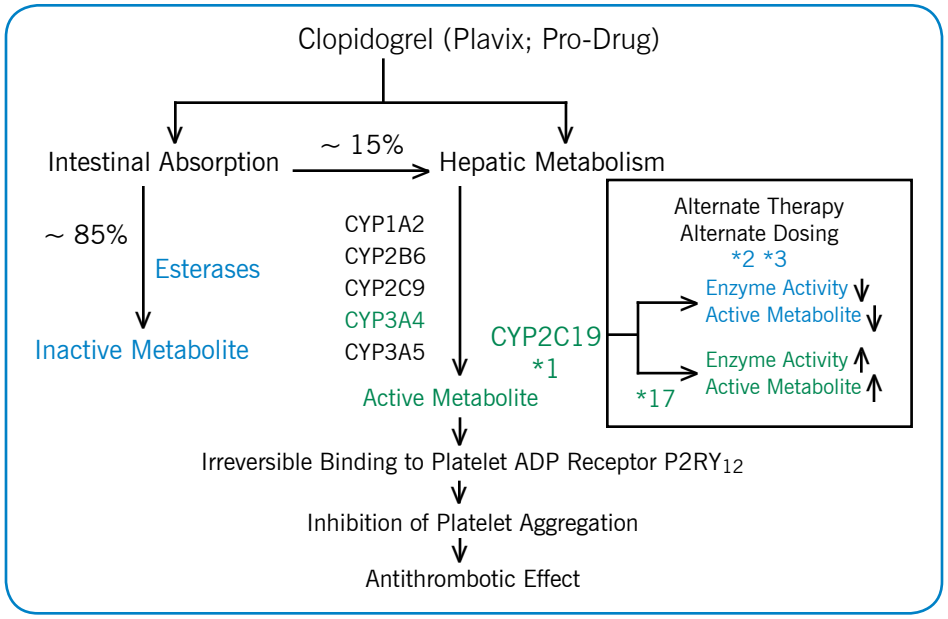

FIGURE 1. METABOLISM AND MECHANISM OF ACTION OF CLOPIDOGREL

4. Prevalence of *CYP2C19* gene variants differs depending on racial and 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.
5. This genotyping assay should be used in conjunction with the aspirin/clopidogrel aggregation assay as a functional screen to determine platelet response to clopidogrel.
6. 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 (AutoGenomics 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 fluorescent labeled dCTP and hybridization on to a micro-array 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 that may influence drug effect and metabolism. *CYP2C19* variant alleles are important in the metabolism of drugs other than clopidogrel including but not limited to anticonvulsants, antidepressants, antiulcer and antimalarial drugs, and proton pump inhibitors. Co-administration of drugs metabolized by CYP2C19 may increase or decrease the CYP2C19 activity. 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.

References

1. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet*. 2009;373:309-317.
2. Desta Z, Zhao X, Shin JG, Flockhart DA (2002). "Clinical significance of the cytochrome P450 2C19 genetic polymorphism". *Clin Pharmacokinet*. 2002;41:913-58.
3. Ellis KJ, Stouffer GA, McLeod HL, Craig CR. Clopidogrel pharmacogenomics and risk of inadequate platelet inhibition: US FDA recommendations. *Pharmacogenomics*. 2009;10:1799-1817.
4. Flockhart, DA (2007). "Drug Interactions: Cytochrome P450 Drug Interaction Table". Indiana University School of Medicine. http://medicine.iupui.edu/flockhart/table.htm. Accessed on April 6, 2011.
5. 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;360:354-362.
6. Sangkuhl K, Klein TE, Altman RB. Clopidogrel pathway. *Pharmacogenetics and Genomics*. 2010;20:463-465.
7. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, Damcott CM, Pakyz R, Tantry US, Gibson Q, Pollin TI, Post W, Parsa A, Mitchell BD, Faraday N, Herzog W, Gurbel PA. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009;302:849-857.
8. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, Morath T, Schömig A, von Beckerath N, Kastrati A. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation*. 2010;121:512-518.
9. 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;360:363-375.

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Test Overview

Test Name	Genetic Test of Cytochrome P450 2C19 (CYP2C19) for Clopidogrel Therapy
Methodology	Multiplex PCR and array hybridization assay
Specimen Requirements	Testing Volume/Size: 3 mL; Type: Whole blood; Tube/Container: EDTA (Lavender); Collection Temperature: Refrigerated
Reference Range	An interpretive report will be provided.
Ordering Mnemonic	2C19CL
Billing Code	88362
CPT Codes	83891, 83900, 83901, 83914 x6, 83912

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