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Genetic Variations in a Cytochrome P450 Enzyme and the Effects on Clopidogrel Bioactivation and Metabolism

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Introduction
Over the last 11 years, physicians have prescribed clopidogrel (Plavix<sup>®</sup>) to more than 100 million people. Clopidogrel is commonly used to prevent thrombotic events in patients who have recently experienced a myocardial infarction (MI) or cerebral vascular accident (CVA) and in those who have established peripheral arterial disease (PAD). Given that clopidogrel is an orally administered prodrug, its efficacy is dependent on its bioactivation via hepatic metabolism. Cytochrome P450 enzymes, especially CYP2C19, are essential for the conversion to its active form. Consequently, any variability in CYP2C19 can significantly impact the bioactivation of clopidogrel, thereby influencing its efficacy. As a result, the approved Plavix<sup>®</sup> product labeling cautions prescribers that reduced effectiveness is seen in patients with impaired CYP2C19 function.

Black Box Warning
In March 2010, the United States Food and Drug Administration (FDA) added a black box warning to the clopidogrel labeling to alert health care professionals that the drug may be less effective in a certain population of patients who are unable to convert it to the active form. The black box warning states, “Clopidogrel hydroquinone sulfate effectiveness is dependent on its activation to an active metabolite by CYP2C19. In patients who are CYP2C19 poor metabolizers, clopidogrel at recommended doses forms less of that metabolite and has a smaller effect on platelet function. Compared with normal metabolizers, poor CYP2C19 metabolizers with acute coronary syndrome or undergoing percutaneous coronary intervention treated with clopidogrel at recommended doses exhibit higher cardiovascular event rates. Tests are available to identify a patient’s CYP2C19 genotype; these tests can help determine therapeutic strategy. Consider alternative treatment or treatment strategies in CYP2C19 poor metabolizers.” By encouraging the use of genetic tests, pharmacists can help in identifying patients who are poor metabolizers of clopidogrel, thus promoting the personalization and improved efficacy of clopidogrel therapy.

Normal Clopidogrel Metabolism
Platelet aggregation is a normal response to tissue injury, adenosine diphosphate (ADP) released from platelets activates neighboring platelets by binding to the P2Y<sub>12</sub> receptor, thus promoting platelet aggregation. The active form of clopidogrel inhibits this platelet aggregation by irreversibly binding the P2Y<sub>12</sub> receptor and inhibiting the full activation of the glycoprotein IIb/IIIa adhesion receptors on platelets (Figure 1).
Pharmacogenomics

Genetic Variations in a Cytochrome P450 Enzyme and the Effects on Clopidogrel Bioactivation and Metabolism

The CYP2C19 gene, *2, *3, *4, *5 and *17. The most common variants are the first four, while the CYP2C19*17 variant is associated with the ultra-rapid metabolizer phenotype.

Clopidogrel Resistance Due to Genetic Variants

Three main factors have been identified that contribute to the overall resistance of clopidogrel displayed in some patients; these include differences in gastrointestinal (GI) absorption, platelet receptor abnormalities, and variations in hepatic metabolism. The GI absorption of clopidogrel is dependent on two main features: the activity of GI esterases and P-gp efflux pumps. Polymorphisms in the genes that code for P-gp efflux pumps have been associated with an increase in the GI efflux of clopidogrel. Platelet receptor abnormalities, or polymorphisms in the genes coding for the glycoprotein IIb/IIIa or the P2Y12 receptor, can cause decreased metabolite binding and subsequent decreases in platelet aggregation.

The factor with the greatest impact on the resistance to clopidogrel is the genetic variations in the CYP isoenzyme pathways of hepatic metabolism. The polymorphism identified to have the most significant impact is that of the CYP2C19 gene. It is estimated that 33-50 percent of the population has a variant form of the CYP2C19 gene. Full function of this gene, associated with the wildtype *1 allele, is required in both steps of clopidogrel metabolism: first for conversion of clopidogrel to 2-oxo-clopidogrel, and then for metabolism of 2-oxo-clopidogrel to the active compound (Figure 1). Many studies have linked variations in CYP2C19 to an increased risk of death or nonfatal MI or CVA as well as a greater occurrence of in-stent thromboses.

The FAST-MI studies linked genetic variation in the gene coding for the given enzyme, especially the *2 allele, to significantly increase the risk of death, stroke, MI, revascularization and in-stent thrombose, expression of any two of the variants associated with loss of function, particularly the *2, *3, *4, or *5 alleles, nearly doubled the risk of death or nonfatal MI or stroke within one year (p=.045). The PRINC Trial demonstrated that if identified, individuals with genotypes indicative of poor response to clopidogrel, particularly those expressing the *2 or *4 alleles, could experience an increased degree of platelet inhibition if their dosing was modified from what is currently considered to be the standard dose. The study showed that while wildtype carriers had better platelet inhibition two hours after a 600 mg loading dose compared to inhibition in *2 and *4 individuals (p=.026), individuals with the *2 and *4 alleles had greater inhibition than wildtype individuals with a 1,200 mg loading dose at four hours (p=.002). Additionally, *2 and *4 individuals responded better than wildtype individuals with a 150 mg maintenance dose compared to the standard 75 mg maintenance dose (p=.042).

By taking into account an individual's age, BMI, lipid levels and CYP2C19 genotype, approximately 22 percent of the variation of clopidogrel response can be explained. This leads to the conclusion that assessment of the CYP2C19 genotype may prove to be a useful clinical tool for helping health care providers choose the most efficacious antiplatelet therapy and dosing regimen for a given patient.
Non-genetic Testing
Phenotyping can be performed to categorize the functional aspect of a particular drug metabolizing enzyme. This is not a genetic test; rather, it involves the patient consuming a probe drug that utilizes the enzyme pathway of interest. Measurements are made using the probe drug and an inactive metabolite, and these measurements are then converted into a ratio that serves as the "metabolic ratio." This ratio is used to determine the metabolic rate that allows an individual to be characterized as an EM, PM or IM. These probe tests can be performed using a single probe, or a "cocktail" of several probe drugs can be utilized to assess multiple enzyme pathways concurrently. Before a "cocktail" is used, it must be determined that no drug interactions exist between any of the probes used to avoid confounding the resultant metabolic ratios.

The phenotypic categories can be used to quantify how a patient will metabolize a drug utilizing that certain enzyme. The effect of being a PM depends on whether the drug in question is pharmacologically active or a prodrug. In the case of clopidogrel, a PM will experience a decrease in bioavailability of the active form of the drug. Phenotyping may be difficult to perform clinically because the measurements are not taken until a number of hours after drug administration, therefore making this type of testing more suitable for a research setting.

An additional testing option is the proprietary VerifyNow-P2Y12 test, a point-of-care assay that was developed by Accutronics. A small blood sample is taken, and an ACP agonist is used to stimulate platelet aggregation. The test then measures how well platelet aggregation is impaired by clopidogrel. Thrombin receptor agonists are also used to approximate the patient's baseline platelet aggregation.

Genetic Testing
Molecular genetic testing entails examining the gene or genes that code for an enzyme to see which alleles are present. There are several genetic tests available to investigate how well an individual will metabolize clopidogrel. Two available tests are AmpliChip™, developed by Roche Diagnostics, and Quest Diagnostic's AccuType™ CP. These two genetic tests involve taking a patient's blood sample and testing for variants in the CYP2C19 genes using polymerase chain reactions (PCRs), which is a method to make many copies of the genes so it can be more easily measured. A computer program then analyzes the PCR data and determines the genotype and corresponding phenotype of the patient.

The AmpliChip™ test is designed to be able to have the results available in an eight-hour period. However, the time it takes for a patient's blood to be drawn and tested and the results to be returned varies depending on the lab and test used. In order for these genetic tests to become more clinically useful in clopidogrel therapy, technology must be developed to make test results available prior to dosing. Research currently is being done on a technology that involves the use of a fluorescence-based assay that measures an energy exchange to distinguish the target DNA sequence that codes for the specific enzyme. The goal is to develop a portable, low-cost and real-time assaying platform that could potentially be used for on-site genetic testing.

Advantages and Disadvantages of Genetic Testing
The costs of different genetic tests vary depending on the test and lab being used but generally range from $250 to $500. These tests may be covered by a patient's insurance plan, though many insurance companies consider them to be "investigational, experimental or unproven." Due to evidence that genetic variations in the CYP2C19 gene are associated with increased risks for the occurrence of adverse cardiovascular events and in-stent thromboses, using pharmacogenomics and genetic testing with clopidogrel therapy presents a real opportunity to decrease health care costs despite the added costs of the testing itself. The use of genetic tests can help the clinician identify and prevent situations in which patients undergo clopidogrel treatment that is rendered ineffective due to their genetic makeup. However, these tests also may identify the need for a treatment that is likely to be more effective but also more costly. The clinical application of this technology also has some drawbacks, including the invasive nature of these genetic tests, which require blood to be drawn. Moreover, including a genetic test before prescribing clopidogrel to a patient will increase turn-around time for a patient to receive the appropriate anti-platelet therapy.

Using genetic tests to determine if a patient has any polymorphisms in the gene for CYP2C19 may aid in individualizing and optimizing clopidogrel therapy. In a study done at the Sinai Hospital in Baltimore, MD., those with the CYP2C19*2 gene mutation demonstrated no difference in baseline platelet aggregation compared to those without the "loss of function" allele. However, those with the mutation demonstrated greater residual platelet aggregation after clopidogrel therapy. After one year of follow-up, those with the CYP2C19*2 genotype had higher rates of cardiovascular events compared to those without the mutation. This demonstrates the advantages of using genetic testing in conjunction with clopidogrel therapy.

Conclusion
Pharmacists can play a key role in increasing awareness and accessibility of genetic testing, especially in the area of clopidogrel prescribing and dosing. It is the responsibility of pharmacists to ensure that patients understand the benefits of these genetic tests. In conjunction with pharmacist-run anti-coagulation clinics, on-site genetic testing and subsequent clopidogrel dosing by a pharmacist could become a standard made available to patients. Pharmacists, in both community and hospital settings, may recommend to a prescriber an increased dose of clopidogrel or a change in therapy to an entirely different medication for patients who have been deemed PMs according to results of their genetic testing. Using data presented in the PRINC trial, pharmacists can advocate for patients who are classified as PMs of clopidogrel, based on their genotype concerning the CYP2C19 gene, to be treated with higher loading and maintenance doses. This will allow them to see platelet inhibition comparable to outcomes observed with the standard dosing in EMs. Such recommendations could consequently improve clinical outcomes for patients who would otherwise see no benefit from this pharmaceutical therapy; however, patients can only benefit from this type of intervention if their genotype can be quantified via genetic testing for variants in the CYP2C19 gene. The application of these genetic test results is an area of practice that pharmacists can embrace as part of the movement toward increased patient counseling and medication therapy management. The use of genetic testing in the prescription and dosing of...
clopidogrel presents an opportunity for pharmacists in most practice settings to expand the degree of care provided to patients by encouraging the personalization of clopidogrel therapy. Rather than adhering to the current "one size fits all" method of prescribing, pharmacists have the opportunity to take a more proactive approach to customize antiplatelet therapy based on a patient's individual needs and significantly increase the efficacy of patient treatment with clopidogrel.

References
Assessment Questions

1. Which of the following are utilized in genetic testing?
   a. AmpliChip™
   b. AccuType™
   c. VerifyNow-P2Y12
   d. A and B

2. AmpliChip™ uses ______ to determine genotype.
   a. Probe drug
   b. PCR
   c. ADP agonist
   d. None of the above

3. Which factor has been identified to contribute the most to variation in response to clopidogrel therapy?
   a. Variations in the CYP2C19 enzyme
   b. Platelet receptor abnormalities
   c. Activity of GI esterases
   d. Activity of P-gp efflux pumps

4. What is released from platelets during a normal response to tissue injury?
   a. PLC
   b. ATP
   c. ADP
   d. cAMP

5. What percentage of a normal orally administered dose of clopidogrel is metabolized in the liver to the active form?
   a. 60 percent
   b. 50 percent
   c. 25 percent
   d. 15 percent

6. An individual with two "loss of function" alleles is characterized as which type of metabolizer?
   a. Extensive metabolizer
   b. Intermediate metabolizer
   c. Poor metabolizer
   d. Normal metabolizer

7. According to the conclusions of the TRITON-TIMI study, all of the following are true for carriers of the CYP2C19*2 variant EXCEPT:
   a. Decreased risk of in-stent thrombosis
   b. Increased risk of death from cardiovascular events
   c. Increased risk of non-fatal MI or CVA
   d. Two of the above

8. Which are ways that pharmacists can be involved in genetic testing and clopidogrel dosing?
   a. Ensure that patients are aware of testing availability and benefits.
   b. Provide clinics or on-site genetic testing for patients.
   c. Recommend a change in dosage or therapy if indicated by test results.
   d. All of the above

9. According to the PRINC study, which options are available to optimize therapy for poor metabolizers of clopidogrel?
   a. Longer course of therapy
   b. Higher loading and maintenance doses
   c. Recommend switching to a different therapy
   d. B and C

10. All of the following are benefits of genetic testing EXCEPT:
    a. Prevention of futile therapy
    b. Rapid time frame to achieving appropriate clopidogrel therapy
    c. Decreased overall health care costs
    d. Decreased clinical complications

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<td>The program objectives were clear.</td>
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<td>Describe the variables that account for differences in response to clopidogrel therapy and which accounts for the most variation in response.</td>
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<td>Explain the various testing methods available to detect a variation that can result in decreased clopidogrel response.</td>
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<td>Discuss what constitutes a poor metabolizer and what are the risks associated with this phenotype.</td>
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<td>Explain the pharmacist’s role in advocating for and providing access to genetic testing.</td>
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Answers to Assessment Questions - Please Circle Your Answer


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