Clinical Chemistry 61:10
1235–1240 (2015)

Clopidogrel and CYP2C19: Pharmacogenetic Testing Ready for Clinical Prime Time?

Moderators: Michael J. Knauer[1] and Eleftherios P. Diamandis[2*]
Experts: Jean-Sebastien Hulot,[3] Richard B. Kim,[4] and Derek Y.F. So[5]

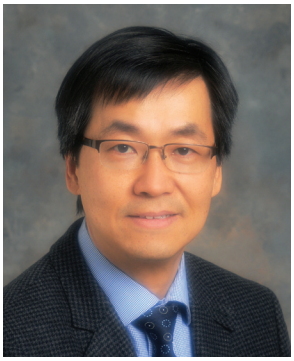
Dual antiplatelet therapy with clopidogrel and aspirin has become the mainstay of therapy for patients with acute coronary syndrome (ACS)[6] undergoing percutaneous coronary interventions (PCI). Many pharmacokinetic and pharmacodynamic studies have demonstrated substantial interindividual variation in antiplatelet response with clopidogrel, a significant proportion of which is explained by the variation in plasma concentrations of the clopidogrel active metabolite. Clopidogrel is a prodrug that requires bioactivation by the highly polymorphic enzyme CYP2C19 to form the active metabolite. The growing body of literature has implicated the loss-of-function cytochrome P450, family 2, subfamily C, polypeptide 19 variant (*CYP2C19*2*)[7] variant with an increased risk of major cardiovascular events. This evidence prompted the US Food and Drug Administration (FDA) to implement a boxed warning on the clopidogrel label describing the relationship between *CYP2C19* pharmacogenetics and drug response, emphasizing the diminished effectiveness in CYP2C19 poor metabolizers.

CYP2C19 pharmacogenetic testing is currently available to guide antiplatelet therapy; however, there are challenges with implementing pharmacogenetic-guided therapy in clinical practice. In this Q&A, 3 experts discuss the current state, challenges, and future direction of *CYP2C19* pharmacogenetic testing for clopidogrel therapy.

Can you briefly summarize the latest developments and evidence for pharmacogenetic testing in patients on clopidogrel therapy?

Jean Hulot: Clopidogrel is a prodrug that requires hepatic bioactivation to generate an active metabolite with

antiplatelet properties. The CYP2C19 enzyme is directly involved in this bioactivation process but its activity is genetically determined. A common loss-of-function genetic variant (named *CYP2C19*2*; c.681G>A; rs4244285) is associated with reduced formation of the clopidogrel active metabolite and reduced pharmacodynamic response to the drug, thus resulting in high on-treatment platelet reactivity and more frequent adverse cardiovascular events. This effect is particularly observed in homozygous carriers of the mutated allele (so-called poor metabolizers), who represent 3%–4% of Caucasian patients. The FDA-approved drug label warns that CYP2C19 poor metabolizers may have diminished effect of the drug and suggests genetic testing to identify these patients. Finally, clopidogrel remains the most frequently prescribed P2Y12 antagonist.



Richard Kim: The clinical relevance of pharmacogenetic testing, particularly with regard to CYP2C19 and clopidogrel, has been well documented and continues to suggest clinical merit and relevance. Specifically, there is little doubt with regard to the importance of CYP2C19 in the 2-step

[1] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [2] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; Department of Clinical Biochemistry, University Health Network; Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; [3] Director of Pharmacogenomics & Personalized Therapeutics, Cardiovascular Research Center, Icahn School of Medicine at Mount Sinai School, New York, NY; [4] Professor of Medicine, Physiology and Pharmacology, and Oncology, Wolfe Medical Research, Chair in Pharmacogenomics, Department of Medicine, Schulich School of Medicine and Dentistry, Western University, London, Ontario, Canada; [5] Program Director, Adult Interventional Cardiology, University of Ottawa Heart Institute, Ottawa, Ontario, Canada.

* Address correspondence to this author at: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, 6th Floor, Rm. 6–201, Box 32, 60 Murray St., Toronto, Ontario, Canada M5T 3L9. Fax 416-619-5521; e-mail ediamandis@mtsinai.on.ca.

Received June 19, 2015; accepted July 6, 2015.

[6] Nonstandard abbreviations: ACS, acute coronary syndrome; PCI, percutaneous coronary intervention; FDA, US Food and Drug Administration; MACE, major adverse cardiac events; AHA, American Heart Association; CPIC, Clinical Pharmacogenetics Implementation Consortium; POC, point of care; POPular, Patient Outcome after primary PCI; TAILOR-PCI, Tailored Antiplatelet Therapy following PCI.

[7] Human genes: *CYP2C19*, cytochrome P450, family 2, subfamily C, polypeptide 19; *ABCB1*, ATP-binding cassette, sub-family B (MDR/TAP), member 1.

bioactivation process to generate the clopidogrel active metabolite. There are now evidence and guidelines for implementing *CYP2C19* genotyping, particularly for patients who undergo PCI for ACS. There remains some controversy relating to the overall clinical relevance of *CYP2C19* genotyping for clopidogrel response for other clinical conditions where clopidogrel is sometimes prescribed, such as in the setting of atrial fibrillation for stroke prevention.

Derek So: CYP2C19, an isoenzyme in the cytochrome P450 system, has been suggested to be integral in the biotransformation of clopidogrel. Loss-of-function *CYP2C19* alleles, carried by up to 30% of individuals, have been associated with increased adverse outcomes in clopidogrel-treated patients with ACS undergoing PCI. The *CYP2C19*2* allele (rs4244285) constitutes 95% of all loss-of-function alleles for *CYP2C19*, while the *CYP2C19*3* allele (rs4986893) accounts for another 1% among those of Western European descent. In Asians, the **3* allele is found in up to 15% of the population. A metaanalysis of 9685 patients confirmed that carriers of *CYP2C19* loss-of-function alleles suffer from an increased incidence of major adverse cardiac events (MACE) (hazard ratio, 1.55) and stent thrombosis (hazard ratio, 2.67) compared to noncarriers. Novel inhibitors of P2Y12-mediated platelet inhibition, prasugrel and ticagrelor, provide enhanced platelet inhibition compared to clopidogrel; however, these agents are associated with an increased risk of major bleeding. An ideal strategy would be selective administration of more potent P2Y12 agents to patients at high risk for ischemia. Meanwhile patients at a low risk can remain on clopidogrel and can be spared bleeding risks and costs associated with more potent agents.

What is the current status of adopting pharmacogenetic testing into routine clinical practice when prescribing clopidogrel?

Jean Hulot: The use of pharmacogenetic testing in routine clinical practice remains limited mainly because the genetic information is not available at the time of prescription. A couple of local initiatives have recently proposed to implement predetermined genetic profiles in the electronic health records of selected patients. The platform thus gives doctors real-time guidance based on the patient's genetic profile.

Richard Kim: Routine pharmacogenetic testing for *CYP2C19* is not recommended by the American Heart Association (AHA) 2012 guidelines. Accordingly, there has not been much progress in translation of CYP2C19 testing into routine clinical practice in the community. However, it should be noted that a number academic institutions have implemented hospital-wide reporting of *CYP2C19* genotypes in their electronic medical records to facilitate provider order entry decision support. The recent approval of other P2Y12 antagonists such as prasugrel and ticagrelor, which are not significantly affected by pharmacogenetic factors, has meant that healthcare providers have the option of bypassing the need or consideration for *CYP2C19* genotyping by prescribing one of these agents rather than clopidogrel.

Derek So: Currently, clinical guidelines do not endorse adoption of routine pharmacogenetic testing for prescription of clopidogrel. However, the guidelines do suggest the consideration for use among special groups, such as those with recurrent ACS despite ongoing treatment with clopidogrel; accordingly, it has been given a class IIb recommendation in current American College of Cardiology/AHA guidelines, with the intention of it to be reassessed after better clinical evidence becomes available. Of note, the Spartan RX CYP2C19 test system and the Nanosphere Verigene CYP2C19 Nucleic Acid Test are FDA approved for in vitro testing and reimbursement via Medicare (CPT code 81225). Therefore, its use on a clinical basis is available for physicians in the United States.

Should all patients on clopidogrel undergo pharmacogenetic testing, and if not who should be tested? What polymorphisms should be genotyped? How should the genotypes be determined and interpreted?

Jean Hulot: There is no evidence supporting a systematic screening for patients requiring clopidogrel. Current data rather indicate that *CYP2C19* loss-of-function alleles (mainly *2) are associated with higher cardiovascular outcomes in patients presenting with an ACS and requiring PCI. *CYP2C19* loss-of-function alleles have also been associated with a higher risk to develop coronary stent thrombosis.

Most of the studies have reported an increased cardiovascular risk in carriers of *CYP2C19*2* receiving clopidogrel. The influence of other loss-of-function alleles is more controversial, mainly because of their low frequency. In addition, the risk is well demonstrated in *CYP2C19*2* homozygotes but remains a matter of debate in heterozygotes. A large variability in clopidogrel response remains in *CYP2C19*1/*2* and the appropriate adjustment strategy in these patients remains unclear.

Richard Kim: Currently, the strongest evidence exists for *CYP2C19* genotyping in the setting of ACS patients who undergo PCI. This would be a group of patients who merit preemptive genotyping. The recommendation for interpretation of the *CYP2C19* genotype for clopidogrel is clearly outlined in the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline. As genotyping-associated costs continue to decline, and I suspect in the future most patients will have *CYP2C19* genotyping (or even NextGen sequencing) carried out as a part of a much larger pharmacogenetic panel, the utility of such test results will be in linking the results and recommendation at the time of initiation of antiplatelet therapy. Although the cost of newer agents such as ticagrelor is higher than that of clopidogrel, it is possible that such price gaps may narrow substantially in the coming years. However, for the foreseeable future, cost difference is likely to be a major consideration to *CYP2C19* genotyping since a generic version of clopidogrel was approved by the FDA in 2012. Indeed, the cost of a 30-day supply of clopidogrel appears to be near $12.00 at various pharmacies in the United States while the list price for a 30-day supply of ticagrelor is nearly $285, although likely much cheaper when using manufacturer coupon or assistance programs.

In terms of functional consequences, the common *CYP2C19*2* allele encodes for a complete loss of function allele and is the most common and clinically relevant genotype for those of Caucasian descent. It should be noted that in other racial groups, such as Asiatics, in addition to *CYP2C19*2*, the *CYP2C19*3* allele, another complete loss-of-function genetic variation is also observed with sufficient frequency. Therefore, interpretation of genotypes should include caveats that note the current genotyping tests only rule in or out common variants and being genotyped and being labeled as *CYP2C19*1/*1* does not guarantee the absence of other more rare functional genetic variation(s) in the patient's DNA.

Derek So: As mentioned above, routine pharmacogenetic testing of all patients with ACS or those undergoing PCI cannot be supported by present evidence. However, for at-risk patients, such as those with recurrent events, a better understanding of the mechanisms underlying treatment failure would be of benefit for a personalized strategy.

If testing were to occur, the *CYP2C19* loss-of-function alleles have the strongest evidence for association to ischemic complications, with the *CYP2C19*2* and *CYP2C19*3* variants comprising over 95% of loss-of-function alleles. The ATP-binding cassette, subfamily B (MDR/TAP), member 1 (*ABCB1*) gene encodes an intestinal efflux pump and affects clopidogrel gut uptake. Homozygous carriers of the *ABCB1* c.3435C>T (rs1045642) variant have an increased propensity for ischemic outcomes after ACS when treated with PCI and clopidogrel; accordingly, positioning it as a possible variant for screening. Lastly, bleeding risk is also an important consideration in the management of these patients. The *CYP2C19*17* is a gain-of-function allele and has been associated with increased bleeding among carriers treated with clopidogrel. A better understanding of the interplay of these potential target variants would enable future application of genetic data towards personalized antiplatelet treatment.

How does turnaround time factor in when determining a patient's CYP2C19 genotype? What should the target turnaround time be and is there a need for rapid genotyping or point-of-care genotyping assays?

Jean Hulot: So far, the use of pharmacogenetic testing in routine practice has been largely limited by the long turnaround time. Clopidogrel is likely prescribed in an emergency setting and any therapeutic adjustment should be decided in a very limited time frame. The pharmacogenetic information should eventually be available at the time of prescription. Point-of-care (POC) genotyping assays might be part of the solution.

Richard Kim: *CYP2C19* genotyping turnaround time is likely of most relevance in the setting of ACS, when an antiplatelet agent such as clopidogrel is likely to be prescribed. Indeed, in this population of patients, knowing the genotype with a rapid turnaround time may be particularly helpful in terms of selecting clopidogrel vs other agents. It is difficult to know what the target turnaround time should be, but within 24 h would be desirable. Indeed, a number of POC genotyping technologies, including that of Spartan and Nanosphere Verigene systems, are available.

Derek So: As my colleagues indicated, the target patients for this technology are those undergoing PCI or those with ACS. Often management decisions for these patients must be made in an expedient manner since potential delays may incur risk for ischemic or bleeding complications. In patients undergoing PCI, the risk for stent thrombosis is rare, but potentially devastating. This complication occurs most often within the first several days after stent deployment; therefore, the potential use of genetic testing would ideally be at the time of antiplatelet initiation. Consequently, the most prudent argument for genetic testing is a POC device that would enable very quick turnaround of data, ideally within the first hour. Even with this scenario, ACS patients may require initial treatment with a more potent agent because delay to angiography for proper diagnosis and management may incur potential complications.

Currently available devices have capabilities to perform testing within the first hour. Subsequent generations of this technology may enable determination of genetic carrier status within 30 min of test initiation. While it may be argued that conventional laboratory testing may be used for patients undergoing elective PCI, there are still logistical issues that may cause delays. Therefore, if novel POC systems may provide sensitivity and specificity equal to that of conventional genotyping and at comparable costs, then it would be argued that POC testing is the ideal modality for this group of patients.

What are the issues or challenges faced with pharmacogenetic testing, interpretation, and reporting to the ordering physician?

Jean Hulot: Pharmacogenetic testing requires appropriate consent from the patient and specific authorizations to be performed and interpreted. The pharmacogenetic information will be used to adjust the patient therapies with the hope of providing a more personalized treatment strategy that improves the efficacy while reducing safety issues. On the other hand, any errors in genotyping or interpreting the results might lead to inappropriate treatment adjustment that could prove harmful. Presently, new P2Y12 antagonists are available and can be used in place of clopidogrel. The efficacy of these drugs is *CYP2C19*-independent but they are associated with a higher risk of bleeding.

Richard Kim: Although there are a number of challenges for the field of pharmacogenetics, for *CYP2C19* genotyping in the setting of ACS and PCI, other than rapid turnaround time and accuracy of the test results, there is sufficient evidence to support the use of *CYP2C19* genotyping information in selecting the most appropriate antiplatelet agent. Before the availability of newer agents such as ticagrelor, substantial research effort had been focused on identifying the best dose(s) of clopidogrel for those who carry a variant allele. However, using *CYP2C19* genotype to guide antiplatelet drug selection makes clinical recommendation simpler and easier for requesting physicians to prescribe.

Derek So: Major obstacles to the use pharmacogenomics in cardiology include lack of accessibility and the time delay associated with conventional genotyping. Certainly, the advent of POC testing devices overcomes some of these initial challenges and also provides physicians with data directly at the bedside. However, there may be worries about the accuracy of the data compared to conventional laboratory-based genotyping and the legal implications for miscalls. Conversely, conventional laboratory-based genotyping may also be prone to clerical errors in specimen labeling or reporting.

As stated, the lack of prospective trials demonstrating benefit from reduction in clinical endpoints with genotyping is a barrier to the routine application of the technology into antiplatelet selection. Also, genotyping does not exclude all causes for variability in responsiveness to clopidogrel. Factors such as age, body mass index, diabetes, and ACS have all been shown in the literature to affect clopidogrel responsiveness. Therefore, genotyping alone may not prevent all potential causes for ischemic complications. In this context, combined approaches to personalizing antiplatelet therapy may be required in future strategies.

Once the CYP2C19 genotype is determined and interpreted how should the clinician respond? Does routine clinical pharmacogenetic testing make a meaningful impact on clopidogrel therapy and patient outcomes?

Jean Hulot: Two different options have been proposed so far: increasing the clopidogrel dosing regimen or switching to another P2Y12 antagonist. Different studies have shown that increasing the clopidogrel dosing regimen is not able to override clopidogrel resistance, thus favoring the second option. However, one needs to remember that no prospective clinical trial has demonstrated the efficacy of a pharmacogenetic-guided prescription of a P2Y12 antagonist. On the other hand, most of the pharmacological studies have shown that *CYP2C19*2* homozygous carriers are unable to activate clopidogrel and the use of other P2Y12 antagonists in these particular patients should be considered.

Richard Kim: The goal would be to provide specific suggestions with regard to selecting clopidogrel or alternative drugs but leave the ultimate decision to the requesting physician. It is possible, for example, that even when a variant allele is detected and reported, the clinician may choose to continue with clopidogrel if the clinician knows that the patient can only afford the cost of generic clopidogrel. Indeed, since most patients with ACS who undergo PCI are usually placed on low-dose aspirin as well as a P2Y12 antagonist such as clopidogrel, the impact of *CYP2C19* genotype, for the majority of variant carriers (CYP2C19 intermediate metabolizers), may not be as large or clinically significant as those who are true poor metabolizers.

Derek So: On the basis of accumulated evidence linking *CYP2C19* loss-of-function polymorphisms with MACE, the FDA issued a boxed warning for clopidogrel in March of 2010. Among patients in whom testing is felt to be indicated, those who are identified as heterozygous or

homozygous *CYP2C19* loss-of-function carriers are categorized as intermediate or poor metabolizers, respectively. The recommendation by the CPIC is to switch clopidogrel for a more potent agent that is immune to *CYP2C19* influence, such as prasugrel or ticagrelor.

Testing may potentially benefit several groups. From a patient's perspective, those who are identified as carriers of at-risk alleles would derive protection from potentially receiving a drug, which may put them at risk for ischemic complications. Conversely, for noncarriers, there may be the potential benefit of not being exposed to more potent agents, which may put them at risk for bleeding complications. From a payer's perspective, (patients, insurance companies, or national drug plans) there may be substantial cost savings by being able to identify those at risk and selectively treating them with the novel P2Y12 inhibitors, while leaving low-risk patients on clopidogrel, which is generic and several folds cheaper. Also, the potential cost savings to healthcare plans by avoiding treatment of bleeding complications should be considered.

Opponents to routine testing would contend that trials with definitive clinical outcomes would be warranted before substantiating the stated benefits above. However, on an individual patient basis, it could be contended that it will be very difficult not to alter therapy if a patient is identified as a carrier of an at-risk allele. Accordingly, physicians should really weigh potential implications of the test results before ordering genotyping on patients on a routine basis.

Should pharmacogenetic testing be accompanied by therapeutic drug monitoring or platelet function testing in patients taking clopidogrel?

Jean Hulot: There is no evidence supporting this approach, which will remain mainly experimental at that stage.

Richard Kim: This topic has been controversial as well. There is increasing evidence to support that on-treatment platelet reactivity during clopidogrel therapy is greater for those who possess *CYP2C19* variant alleles. Indeed, there are commercially available functional assays of platelet reactivity, such as VerifyNow. However, it is not clear that the cost associated with such functional assays in addition to *CYP2C19* genotyping will result in significantly better clinical decisions with regard to antiplatelet drug selection, beyond *CYP2C19* genotyping.

Derek So: Randomized studies based on platelet function testing have not been able to demonstrate a reduction in ischemic complications in lower-risk patients. However, ongoing studies with novel designs are underway to elucidate this issue further. Genetics do not account for all causes of high on-treatment platelet reactivity. Therefore, it is possible that patients identified as noncarriers of at-risk variants may actually have alternate causes for underresponsiveness to clopidogrel. Ideally, platelet function testing on these individuals may confer additional information and further identify those at potential risk when treated with clopidogrel. Future strategies for individualizing antiplatelet therapy may undertake a combined approach to utilize several methods to identify all at-risk individuals. At present though, the evidence of this type of approach is lacking.

What is the future of pharmacogenetic testing for clopidogrel therapy?

Jean Hulot: The pharmacogenetic influence on clopidogrel response was first reported in 2006 but there is still an underuse of pharmacogenetic testing for clopidogrel therapy. Technical issues explain part of this situation and newly developed POC genotyping assays can address these issues. *CYP2C19* genetic variants are widely recognized as a main determinant of clopidogrel response but they explain a limited proportion of the overall variability. The development of genome-wide techniques will help identify other variants of importance. The combination of multiple variants might provide a more predictive and useful tool in the future.

Richard Kim: I think there is potential for broader pharmacogenetic testing for clopidogrel, driven in part by the current lower cost of generic clopidogrel compared to other agents in the same class, in the setting of increasingly lower genotyping costs. I suspect in the coming years pharmacogenetic testing will be carried out as a panel of tests, rather than having just one gene or genotype, and will be ready for use when drugs such as clopidogrel are prescribed, where the drug choice/dose decision support is built into the electronic medical record system used by the prescribing physician. I suspect that even when the cost of other antiplatelet drugs such as ticagrelor becomes similar to generic clopidogrel, knowing *CYP2C19* genotype may be helpful in assessing adverse reactions; drug-associated dyspnea is more frequent among patients on ticagrelor compared to clopidogrel, and bleeding risk appears to be greater for prasugrel.

Derek So: Currently, the 2 ongoing randomized studies [Patient Outcome after primary PCI (POPular) and Tailored Antiplatelet Therapy following PCI (TAILOR-PCI)] of pharmacogentic testing are anxiously anticipated. TAILOR-PCI will enroll over 5000 patients and will be the largest pharmacogenomic study to date. The results from these studies may then guide the proper adoption of the technology into routine clinical practice and also direct future strategies of personalized antiplate-

let therapy. A key benefit of this technology will be to try to balance ischemia against bleeding outcomes and achieve an ideal therapeutic window. Future studies like POPular and RAPID MANAGE (Reassessment of Anti-Platelet Therapy Using InDividualized Strategies—Modifying Acute CoroNary Syndrome Algorithms Based on Genetic and Demographic Evaluation) will likely use combined ischemic and bleeding outcomes or net adverse clinical events to properly define benefits and harm to patients.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: E.P. Diamandis, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: D.Y.F. So, Spartan Biosciences Inc. Canada.
Research Funding: Eli Lilly Canada; D.Y.F. So, Spartan Biosciences Inc. Canada.
Expert Testimony: None declared.
Patents: None declared.

Previously published online at DOI: 10.1373/clinchem.2015.238105