Review

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Pharmacogenomics

CYP2C19 genotype-guided antiplatelet therapy: promises and pitfalls

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Pharmacogenetic variants can alter the mechanism of action (pharmacodynamic gene variants) or kinetic processes such as absorption, distribution, metabolism and elimination (pharmacokinetic gene variants). Many initial successes in precision medicine occurred in the context of genes encoding the cytochromes P450 (CYP enzymes). CYP2C19 activates the antiplatelet drug clopidogrel, and polymorphisms in the *CYP2C19* gene are known to alter the outcome for patients taking clopidogrel in the context of cardiovascular disease. *CYP2C19* loss-of-function alleles are specifically associated with increased risk for coronary stent thrombosis and major adverse cardiovascular events in patients taking clopidogrel following percutaneous coronary intervention. We explore successes and challenges encountered as the clinical and scientific communities advance *CYP2C19* genotyping in the context of routine patient care.

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Cardiovascular diseases (CVD), which include coronary artery disease (CAD), peripheral arterial disease (PAD) and cerebrovascular disease, remain the leading cause of death worldwide [1]. CVD represents a spectrum of diseases that share similar pathophysiology, and platelets play a pivotal role in this pathophysiology by regulating the development of chronic atherosclerotic plaques and triggering molecular cascades that lead to the progression of arterial thrombosis in response to atherosclerotic plaque rupture [2]. Thus, antiplatelet therapy is considered a cornerstone in the treatment and prevention of CVD. A list of common antiplatelet drugs used in CVD treatment and prevention is outlined in Table 1.

Aspirin was one of the first antiplatelet agents to show benefit in patients with CVD. Through irreversible inhibition of COX enzymes, particularly COX-1, aspirin blocks the production of thromboxane A2, which is a vasoconstrictor and a stimulant for platelet aggregation. Aspirin thereby reduces thrombus formation and CVD events. While the use of aspirin as monotherapy in the context of primary prevention remains a topic of ongoing debate [3–5], aspirin has been shown to have great efficacy in secondary prevention of cardiovascular diseases, especially when combined with other antiplatelet medications [6]. In patients with known CVD, aspirin reduces the risk of nonfatal myocardial infarction and stroke by 26 and 25%, respectively, and it further reduces all-cause mortality by 13% [7].

Adenosine diphosphate (ADP) stimulates platelet aggregation *in vivo*, and P2Y12 antagonists (purinergic receptor P2Y, G-protein-coupled 12 adenosine diphosphate receptor antagonists) are potent inhibitors of platelet aggregation. Ticlopidine was among the first clinically available P2Y12 antagonists, but its use was associated with hematologic adverse events such as neutropenia and thrombotic thrombocytopenic purpura [8]. Newer, safer P2Y12 antagonists eventually emerged in routine clinical practice, including clopidogrel and prasugrel. The clinical benefit of adding clopidogrel to aspirin therapy in acute CAD has been demonstrated in numerous large, multicenter, randomized controlled trials [9–11]; however, the combination has failed to show superior clinical efficacy, compared with aspirin alone, within stable CAD.

Acute coronary syndromes are now often treated by percutaneous coronary intervention (PCI), and prescription of dual antiplatelet therapy (DAPT) with a P2Y12 antagonists such as clopidogrel plus aspirin has become

Table 1. List of antiplatelet medications along with their mechanism and forms of metabolism.

Drug	Route	Mechanism of action	Metabolism
Aspirin	Oral	COX inhibitor, nonselective	Hydrolysis by tissue esterases
Cilostazol (Pletal)	Oral	Phosphodiesterase III inhibitor	Oxidation by CYP3A4 (major) and CYP2C19 (minor)
Dipyridamole (Persantine and Aggrenox)	Oral	Adenosine uptake inhibitor; phosphodiesterase inhibitor	Glucuronidation
Ticlopidine (Ticlid)	Oral	$P2Y_{12}$ inhibitor (irreversible)	*N*-dealkylation and oxidation
Clopidogrel (Plavix)	Oral	$P2Y_{12}$ inhibitor (irreversible)	Oxidation by CYP2C19 (major)†, CYP3A (minor), CYP2B6 (minor)
Prasugrel (Effient)	Oral	$P2Y_{12}$ inhibitor (irreversible)	Oxidation by CYP3A4 (major) CYP2B6 (major), CYP2C9 (minor), CYP2C19 (minor)
Ticagrelor (Brillinta)	Oral	$P2Y_{12}$ inhibitor (reversible)	Oxidation by CYP3A4
Cangrelor (Kengreal)	IV	$P2Y_{12}$ inhibitor (reversible)	Dephosphorylation
Eptifibatide (Integrilin)	IV	Glycoprotein IIb/IIIa inhibitor (reversible)	None
Abciximab (Reopro)	IV	Glycoprotein IIb/IIIa inhibitor (irreversible)	Proteolytic cleavage
Tirofiban (Aggrastat)	IV	Glycoprotein IIb/IIIa inhibitor (reversible)	None

†Primary mechanism for bioactivation.
IV: Intravenous.

the standard of care to maintain stent patency and prevent recurrent coronary events [10,12]. In large populations, however, some patients using DAPT fail to achieve the desired therapeutic outcome from clopidogrel. Newer P2Y12 antagonists such as prasugrel and ticagrelor may deliver improved efficacy in specific patient subsets. In the Trial to Assess Improvement in Therapeutic Outcomes by optimizing platelet Inhibition with Prasugrel Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38), patients receiving prasugrel after PCI for an acute coronary syndrome had a lower incidence of major adverse cardiovascular events (MACE) compared with patients receiving clopidogrel (9.9 vs 12.1%) [13,14]. In the Platelet Inhibition and Patient Outcomes (PLATO) trial, patients receiving ticagrelor after PCI for an acute coronary syndrome also had a lower incidence of MACE compared with patients receiving clopidogrel [15]. Following these landmark trials, both prasugrel and ticagrelor have recently received a class I recommendation for their use in patients who have acute coronary syndromes with or without ST-segment elevation [16,17]. The use of clopidogrel is therefore increasingly being limited to situations where prasugrel and ticagrelor are not well tolerated.

The choice of which P2Y12 antagonist to select within a DAPT regimen after PCI is complex. There is wide variability in cost between clopidogrel, prasugrel and ticagrelor, and each patient's risk for developing toxicity depends upon their prior individual medical history. For example, in some patients, prasugrel has been associated with a higher risk of bleeding than clopidogrel, and prasugrel is therefore contraindicated in patients with a prior history of stroke or transient cerebral ischemia [18,19]. All of these factors – cost, variability in efficacy and variability in toxicity – influence patient adherence. At present, the choice of P2Y12 antagonist is made based upon burden of disease, bleeding risk and clinical factors such as concomitant medication (drug–drug interaction). A key unanswered question has been whether the field of genetics (drug–gene interaction) should be leveraged to further guide clinicians with drug selection.

CYP2C19

In general, P2Y12 antagonists undergo considerable Phase I oxidation by cytochromes P450 (Table 1). Clopidogrel in particular is primarily metabolized by CYP2C19 [20]. Because the *CYP2C19* gene is highly polymorphic, and because clopidogrel is a prodrug requiring bioactivation by this enzyme *in vivo*, the association between *CYP2C19* gene variants and clopidogrel efficacy appears to be clinically actionable. Patients with 'loss of function' *CYP2C19* alleles have lower levels of active clopidogrel metabolites, and these patients have reduced efficacy (less platelet inhibition) when compared with patients with normal alleles [21–26]. Further, loss of function *CYP2C19* alleles are common in patients of all ancestry (Table 2). Approximately 28% of Europeans have at least one loss of function allele, 42% of Africans have at least one loss of function allele and 59% of Asians have at least one loss of function allele [26–28].

The *CYP2C19* gene maps to the long arm of chromosome 10 (10q24) and it encodes a 490-amino-acid protein predominantly expressed in the liver and, to a lesser extent, in the small intestine. The most common nonfunctional

Table 2. Frequency of CYP2C19 enzyme phenotype by major continental race.

CYP2C19 phenotype	European ancestry	African ancestry (sub-Saharan)	Asian ancestry
PM	2%	6%	13%
IM	26%	36%	46%
Normal	40%	30%	38%
RM	27%	19%	3%
UM	5%	3%	0%
Uncertain	<1%	6%	<1%

Frequencies summarized from populations in the Clinical Pharmacogenetics Implementation Consortium. [27].
IM: Intermediate metabolizer; PM: Poor metabolizer; RM: Rapid metabolizer; UM: Ultrarapid metabolizer.

allele, *CYP2C19*2*, has an estimated minor allele frequency (MAF) of approximately 15% in Europeans and Africans, and approximately 30% in Asians [29]. The *CYP2C19*2* allele (681G>A) results in a splicing event that causes premature termination of the protein and leads to a malfunctioning enzyme [30]. Another nonfunctional allele, *CYP2C19*3* (636G>A), is also relatively common in Asian populations (up to 10% are carriers) [27]. Other *CYP2C19* gene variants that lead to loss of function are relatively rare.

It is also important to note that *CYP2C19* gene variants do not account for all of the variability in clopidogrel response. Transcriptional activation of this enzyme is mediated by drug-responsive nuclear receptors such as CAR (NR1I3), PXR (NR1I2) and GRα (NR3C1), suggesting regulation by endogenous hormones and metabolic interference by drugs such as rifampin, ritonavir and dexamethasone [31,32]. Further, pharmacodynamic gene variants in the purinergic receptor and/or in the G-proteins that transduce its molecular signal may also alter outcome for clopidogrel [33]. Combinatorial models including pharmacodynamic genes (e.g., *P2RY12*), and pharmacokinetic genes beyond *CYP2C19* (e.g., *CYP2B6* and *CES1*), may therefore provide even greater power to improve the outcome for antiplatelet therapy [34,35].

Promises

The Clinical Pharmacogenomics Implementation Consortium (CPIC) regularly publishes guidelines for the use of pharmacodynamic and pharmacokinetic drug–gene relationships [36,37]. To date, CPIC has produced 24 guidelines pertaining to 62 medications and 20 pharmacogenes (https://cpicpgx.org/guidelines/). These guidelines provide specific guidance for clinical situations where pharmacogenetic test results are already available; they do not, however, determine when a patient should be genotyped. The decision to genotype should be made by individual patients and clinicians within the context of routine care. Factors influencing the clinical actionability of a drug–gene relationship vary by drug, and they typically include therapeutic index as well as the potential clinical severity of any adverse drug reactions [38]. Examples of drugs that meet these criteria include psychotropic agents, immune modulators, chemotherapeutics and drugs used to modulate hemostasis. Antiplatelet agents specifically have shown great promise in terms of reducing adverse events when a gene-based prescribing approach is applied.

In general, clinician response to pharmacogenetic information will vary depending upon the drug. In some situations, it may be best to adjust the dose based upon genotype – increasing the dose to avoid therapeutic failure or decreasing the dose to avoid toxicity. In other situations, it may be best to change the drug altogether – selecting an alternate agent within the class or moving to a different class of drugs. CPIC guidelines explore each of these approaches, and the specific guidance provided in each guideline varies drug by drug and gene by gene. Essentially, three simple paradigms are emerging. When a patient is identified to have an actionable pharmacogene variant, providers can either change drug, change dose or make no initial change while monitoring more closely (at increased frequency). Each of these paradigms is explored below, specifically for *CYP2C19* genotype and antiplatelet therapy.

Change the drug

Over the past decade, *CYP2C19* genotype has unequivocally been associated with cardiovascular event rate in patients using clopidogrel after percutaneous coronary intervention. Patients using clopidogrel in TRITON-TIMI 38 had a threefold increased risk for coronary stent thrombosis if they were carriers of a *CYP2C19* loss of function allele [24]. These same patients had 50% increase in risk for a combined MACE end point (fatal and non-fatal MI, stroke and cardiovascular death) [24]. This drug–gene relationship therefore appears to be actionable in heterozygotes

(where even one loss of function allele attenuates bioactivation of clopidogrel *in vivo*, placing that patient at threefold increased risk of therapeutic failure).

Subsequent data have indicated that clinicians may be able to avoid therapeutic failure in the context of patients with *CYP2C19* loss of function alleles if carefully selected patients are switched from clopidogrel to prasugrel or ticagrelor. In a genetic substudy of PLATO, ticagrelor reduced MACE events more than clopidogrel in IMs (intermediate metabolizers) and PMs (poor metabolizers), an effect that was larger in IMs and PMs than in patients without a loss of function allele (hazard ratio [HR] of 0.77 vs 0.86, respectively) [15]. More recently, a prospective study of 628 PCI patients in China demonstrated that genotype-guided therapy carried a 55% lower risk of MACE at 12 months (with no difference in bleeding outcomes) when compared with conventional therapy with clopidogrel [39].

Definitive data are now emerging in support of the claim that *CYP2C19* genotype can be leveraged to reduce adverse cardiovascular events by switching PCI patients with a loss of function *CYP2C19* allele from clopidogrel to prasugrel or ticagrelor if they do not have contraindications to these alternate P2Y12 antagonists. The Implementing Genomics in Practice (IGNITE) research network recently replicated findings from TIMI-38 in a practice-based cohort (50% increase in risk for MACE on clopidogrel, in PCI patients with one loss of function *CYP2C19* allele), and they extended those findings to demonstrate that changing the drug (from clopidogrel to prasugrel or ticagrelor) normalized the risk for recurrent MACE events in these patients [24,40].

This strategy of changing drug in select patients (without contraindication) is being widely adopted by organizations such as the Royal Dutch Pharmacists Association Pharmacogenetics Working Group (DPWG) and the US FDA. FDA recently announced a warning on the clopidogrel label recommending consideration of alternative therapies in patients who are *CYP2C19* poor metabolizers as they may not receive the full benefits of the drug. CPIC therefore recommends changing the drug – from clopidogrel to prasugrel or ticagrelor – when most patients with a loss of function *CYP2C19* allele require therapy with a P2Y12 antagonist [30]. In situations where patients with a loss of function *CYP2C19* allele have a contraindication to prasugrel (e.g., prior stroke), or side effects with ticagrelor (e.g., drug-induced dyspnea), *in vitro* data suggest that changing the dose of clopidogrel may be helpful; however, this has not yet been shown to reduce cardiovascular events. To guide clinicians through the implementation of these recommendations within the context of a busy clinical practice day, automated decision support is now being deployed in many commercial electronic medical records [41].

Change the dose

As noted above, there are some situations where changing the drug may not be feasible. Although ticagrelor and prasugrel have been shown to reduce thrombotic events more than clopidogrel in patients with an acute coronary syndrome [15,42], ticagrelor and prasugrel are also associated with higher bleeding risk [15,43]. Further, cost concerns (especially for agents still under exclusivity) may prohibit their use for prolonged therapy. Thus, some patients prefer to stay on clopidogrel despite the presence of a *CYP2C19* gene variant, raising the issue of whether increased doses of clopidogrel (150 mg or 225 mg daily) may be able to overcome the partial decrease in CYP2C19 enzyme activity seen in patients with only one loss of function allele. If successful, an increase in parent drug (doubling or tripling the clopidogrel dose in heterozygotes) could conceivably overcome the enzyme deficit and provide sufficient antiplatelet effect to normalize the risk without incurring the added cost of ticagrelor or the bleeding risk of prasugrel.

Studies done *in vitro* using platelets harvested from patients taking different doses of clopidogrel in the Escalating Clopidogrel by Involving a Genetic Strategy - Thrombolysis in Myocardial Infarction 56 (ELEVATE-TIMI 56) trial have leveraged a platelet activation (VASP) assay to demonstrate that a CYP2C19 enzyme deficit can be partly overcome by dose escalation [44,45]. Mega *et al.* observed a dose-dependent increase in platelet inhibition in both heterozygotes and homozygotes suggesting that this approach may be effective [45]. However, no clinical outcome data have shown that this approach (i.e., changing dose rather than changing drugs) actually reduces the frequency of MACE events. Thus, since data have only shown lab-based efficacy by dose escalation, rather than clinical efficacy (event reduction) by dose escalation, this approach has largely been reserved for situations where either the patient cannot afford the cost of switching drugs, or the patient has other clinical comorbidities that preclude the use of an alternate P2Y12 antagonist, such as age older than 75 years with prasugrel or the development of dyspnea as an adverse drug reaction with ticagrelor [46,47].

Make no change (monitor closely)

Another approach that is often considered with pharmacogenetic data is opting not to change medication, but instead to monitor patients more closely in an attempt to avoid potential adverse outcomes. While such an approach may be appropriate in the context of some drugs metabolized by CYP2C19, it is not advisable for the gene-based prescribing of antiplatelet agents. For example, CYP2C19 oxidizes proton-pump inhibitors (PPIs), tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs). Three SSRIs (citalopram, escitalopram and sertraline) are known to be CYP2C19 substrates, and patients with very low CYP2C19 enzyme activity are at risk of developing long QT and ventricular arrhythmias when taking these SSRIs [48]. In this situation, some clinicians monitor patients on SSRIs using serial electrocardiograms (EKGs) rather than changing the prescription. The key to this approach is the availability of an endophenotype [49].

Conversely, patients at risk of developing coronary stent thrombosis on clopidogrel do not have an easily measured (noninvasive) endophenotype. In patients taking clopidogrel with *CYP2C19* loss of function alleles, there are no simple affordable endophenotypes that can be similarly used for surveillance to monitor the risk of developing coronary stent thrombosis. Serial angiograms would be cost prohibitive and unnecessarily invasive, whereas serial platelet aggregometry has not proven to have sufficient predictive value to determine individual risk for stent thrombosis. Thus, without an endophenotype (such as QT interval for SSRIs), simply monitoring PCI patients with a *CYP2C19* loss of function allele is undesirable. A change in prescription (either changing the drug or changing the dose) is indicated as outlined above.

Pitfalls

While the expansion of gene-based prescribing for antiplatelet agents now has significant momentum in routine practice, several challenges still exist. Rigorous longitudinal analyses are needed prospectively, to define the impact of this approach on medication adherence, quality of care, and cost in an overburdened healthcare infrastructure. Another key challenge related to gene-based drug prescribing has been the issue of pleiotropy, the principle that variation in one pharmacogene often impacts metabolism of multiple other classes of medications [50]. These potential obstacles are discussed below.

Pleiotropy & decision support

CYP2C19 contributes to the metabolism of nearly 10% of all commonly prescribed drugs. As noted earlier, this enzyme oxidizes PPIs, TCAs and SSRIs. Genetic variability in *CYP2C19* has therefore been associated with outcome in the context of many of these drugs [51]. CPIC has published a guideline addressing the relationship between *CYP2C19* gene variants and some SSRIs [48]. Therefore, the clinical and scientific communities need to make sure that any implementation efforts deploying *CYP2C19* genotyping to guide antiplatelet therapy will also provide decision support for patients using SSRIs metabolized by this enzyme. Further, the need for robust decision support (cross talk within electronic medical records that coordinates prescribing among multiple specialties) will almost certainly increase as more outcomes are linked to *CYP2C19* gene variability for additional drug classes (e.g., PPIs). For example, it has been shown that *CYP2C19* genotype predicts healing rates for Barrett's esophagus and eradication rates for *Helicobacter pylori* on triple therapy, in communities where the frequency of *CYP2C19* gene variants is very high [52–54].

Another challenge that complicates implementation of decision support for pleiotropic pharmacogenes has been the issue of whether to deploy decision support for *all* patients that carry a loss of function allele (heterozygotes as well as homozygotes) or to intervene only with patients that have two loss of function alleles (homozygotes). At present, CPIC recommends acting on *CYP2C19* genotype for heterozygotes and homozygotes considering clopidogrel, whereas CPIC recommends acting on *CYP2C19* genotype for homozygotes only in the context of sertraline, citalopram or escitalopram [28,30]. Thus, the importance of robust decision support cannot be overstated. For direct to consumer genotyping, the lack of seamless integration into an electronic medical record may place patients at risk if decision support is not deployed within all clinical contexts where a pharmacogene variant has been proven to be actionable. The convergence of clinical informatics and laboratory genetics will therefore be key to success with *CYP2C19* genotyping.

Operational challenges

Another potential obstacle has been turnaround time when genotyping is ordered reactively within the context of acute care. In this setting, the result may not be readily available by the time a PCI patient leaves the angiography

suite. Even for institutions with an in-house genomics laboratory, turnaround time can be days to weeks. The time window can be even longer if results are sent to an outside lab. Rapid point-of-care genetic testing is being explored at some institutions where these devices appear to return results within 1–6 h [55]. Overall, however, the need to have pharmacogenetic data available in real time is leading to a shift from reactive testing (just in time) to pre-emptive testing (just in case). Pre-emptive genotyping may also lead to cost efficiencies. For example, if a provider orders *CYP2C19* genotyping alone (without any other genetic tests), the out of pocket cost for the patient is likely to exceed $100 (and sequencing the *CYP2C19* gene to find rare variants may cost up to $1000). The cost per gene, however, is driven down markedly if *CYP2C19* genotype is obtained on a larger array containing multiple pharmacogenes. This is leading to an economy of scale, as providers shift to obtaining panels of genes in the context of primary care [56]. As this shift occurs, other potential challenges will develop as well. Simultaneous genotyping that generates decision support for many different classes of drugs holds the potential to overwhelm clinicians with electronic alerts. One proposed solution has been the integration of pharmacists and genetic counselors to deliver genetic information by using a team-based model [50].

Future perspective

CYP2C19 genotyping holds great promise to optimize outcome as clinicians select antiplatelet therapy for individual patients. Emerging data from randomized controlled trials support this claim, specifically for patients undergoing coronary intervention. A recent trial compared genotype-based treatment to usual care in 2488 patients undergoing PCI for an acute coronary syndrome (ACS) at ten European sites [57]. Patients in the usual care arm received prasugrel or ticagrelor. Patients in the genotype-based arm received clopidogrel unless they carried a loss-of-function *CYP2C19* allele, in which case they were switched to prasugrel or ticagrelor. Within this framework, genotype-based treatment was noninferior to usual care with respect to reducing thrombotic events, and genotype-based treatment was associated with a lower incidence of bleeding [57].

Many questions remain, including when to choose prasugrel, and when to choose ticagrelor, as alternates to clopidogrel in patients with loss of function *CYP2C19* alleles. The recent Intracoronary Stenting and Antithrombotic Regimen: Rapid Early Action for Coronary Treatment 5 (ISAR5-REACT) trial results demonstrated superiority of prasugrel compared with ticagrelor in ACS patients undergoing PCI [58]. Patients on prasugrel had fewer MACE events than patients on ticagrelor at 12 months (combined end point of death, nonfatal MI, or nonfatal stroke was 6.9% for prasugrel vs 9.3% for ticagrelor) [58]. Moreover, there was no increase in risk of bleeding with prasugrel compared with ticagrelor. Because prasugrel may be more affordable (due to loss of patent-protected exclusivity in 2017), a very compelling argument is emerging in favor of more wide use of this agent in the absence of specific contraindications (prasugrel is contraindicated in patients with active bleeding, a history of stroke, or age over 75 years).

Guidance is also needed for the use of *CYP2C19* genotype in patients with other forms of vascular disease, beyond CAD. In patients using clopidogrel for peripheral arterial disease (PAD), *CYP2C19* loss of function alleles are associated with diminished platelet response and a poorer prognosis after endovascular treatment [59]. In patients at risk for cerebrovascular disease, the Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) trial showed that the use of clopidogrel plus aspirin (vs aspirin alone) only reduces the frequency of stroke events in the subgroup of patients with normal *CYP2C19* alleles [60]. Much additional work is therefore needed to identify the optimal approach to antiplatelet therapy in patients with transient ischemic attacks (TIA) and stroke.

As these questions are answered, and risk prediction algorithms become increasingly accurate, the convergence of pharmacogenetics and clinical informatics will guide us into the future. Large panels of pharmacogenes being tested pre-emptively in routine care must be linked to automated decision support within electronic medical records in a manner that is efficient and cost effective. The strong association between *CYP2C19* gene variants and outcomes related to antiplatelet therapy is driving this field forward.

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Executive summary

- *In vivo*, CYP2C19 bioactivates the antiplatelet drug clopidogrel.
- Loss of function variants in the *CYP2C19* gene are associated with clopidogrel failure.
- Patients with a single loss of function *CYP2C19* allele are at threefold increased risk for coronary stent thrombosis on clopidogrel when compared with patients with two normal *CYP2C19* alleles.
- Risk for coronary stent thrombosis in patients with *CYP2C19* loss of function alleles can be reduced by switching them from clopidogrel to alternate agents like prasugrel or ticagrelor.

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