

## Pharmacogenetics of Clopidogrel An Unresolved Issue

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### Clinical Case

A 58-year-old male was presented with an ST-elevation inferior myocardial infarction. Emergency medical services administered 325 mg of aspirin and sublingual nitroglycerin en route to the emergency room.

In the emergency room, the patient received 600 mg of clopidogrel and a heparin bolus. Coronary angiography revealed complete occlusion of the distal right coronary artery with thrombus. Intravenous eptifibatid was given at the start of the percutaneous coronary intervention (PCI). Intravascular ultrasound demonstrated significant soft atheroma and ruptured plaque in the distal right coronary artery at the crux. A drug-eluting stent was deployed with an excellent angiographic result.

Postintervention, the patient's chest pain and ECG demonstrated complete resolution of the previous ischemic changes. Approximately 11 hours after being treated in the emergency room, the patient developed recurrent chest pain. He was restarted on eptifibatid and heparin, and repeat coronary angiography demonstrated total occlusion at the origin of the right coronary artery stent (Figure 1).

Thrombectomy was performed, removing huge amounts of atherothrombotic material (Figure 2). Intravascular ultrasound did not demonstrate stent edge dissection or stent malapposition.

A drug-eluting stent with 1-mm overlap with the distal edge of the previous stent was deployed. The patient was felt to have failed clopidogrel therapy and was transitioned to prasugrel therapy. The patient was screened for genetic variants in *CYP2C19*, the gene encoding the cytochrome P450 enzyme CYP2C19 that metabolizes clopidogrel.

### Pharmacogenetic Test Results

A Luminex assay using a polymerase chain reaction was used to test for the presence or absence of *CYP2C19*\*17, \*4, \*8, \*6, \*9, \*3, \*10, \*2, \*7, and \*5 alleles. Genetic testing

revealed a genotype of *CYP2C19* \*1/\*17 or heterozygosity for *CYP2C19*\*17 (rs12248560). Prodrugs, such as clopidogrel, may be activated to the therapeutic metabolite to a greater degree by individuals, who carry the *CYP2C19*\*17 allele.<sup>1</sup> Genetic testing for *CYP2C19* variants did not explain clopidogrel failure or the propensity for stent thrombosis in this patient, paradoxically the *CYP2C19*\*17 allele should have potentially predisposed the patient to increased bleeding complications. Unlike patients with *CYP2C19*\*17 genotype, patients who are CYP2C19 poor metabolizers, that is those who are carriers of *CYP2C19* loss of function (LOF) variant alleles such as \*2 or \*3, have been shown to have a significantly increased risk for stent thrombosis.

### Clopidogrel Pharmacogenetics

Clopidogrel is a prodrug and is metabolized by CYP2C19 into active thiol metabolites by a 2-step oxidative biotransformation process. Only the active metabolite of clopidogrel targets the P2Y<sub>12</sub> receptor for ADP on platelets. The most common LOF variant alleles are *CYP2C19*\*2 and \*3 alleles that result in nonfunctional proteins. The haplotype *CYP2C19*\*2 comprises the variant rs4244285, which is a synonymous single-nucleotide polymorphism affecting the amino acid residue proline at position 227 in exon 5. Although guanine substitution by adenine does not change the proline amino acid, it creates an aberrant splice site, resulting in a premature stop codon.<sup>2</sup> The single-nucleotide polymorphism rs4986893 (G636A) comprising the *CYP2C19*\*3 haplotype results in guanine substitution by adenine and substitution of the TGG codon that encodes tryptophan by TGA, a stop codon in exon 4 resulting in a truncated nonfunctional protein.<sup>3</sup> The minor allele frequency of *CYP2C19*\*2 varies as per ethnic group, being most prevalent in East Asians (29%) and with approximately equal frequency in both the subjects of African (15%) and European (15%) origin.<sup>1</sup> *CYP2C19*\*3 is rare in subjects of European and African ancestry (minor allele frequency <1%); however, it is more common in East Asians (minor allele frequency ≈9%). *CYP2C19*\*2 and \*3 account for ≥99% of the LOF alleles in a multiethnic population<sup>1</sup>

### CYP2C19 Genotype and Clopidogrel Pharmacokinetics

LOF alleles in *CYP2C19* result in lower clopidogrel active metabolite levels.<sup>4,5</sup> The area under the plasma concentration curve during 0 to 24 hours and maximum plasma concentration  $C_{max}$  for the active metabolite of clopidogrel for *CYP2C19*\*2 heterozygote carriers was 54% and 60% that of wild-type subjects.<sup>4</sup> In a separate study by Umemura et al<sup>5</sup> that in addition to *CYP2C19*\*2 included subjects with the *CYP2C19*\*3

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**Figure 1.** Coronary angiogram demonstrating an occluded mid right coronary artery because of stent thrombosis.

allele, area under the plasma concentration curve during 0 to 24 hours was 57% and  $C_{\max}$  was 61% that of wild-type subjects for homozygotes for the active metabolite of clopidogrel.

### CYP2C19 Genotype and Clopidogrel Pharmacodynamics

The presence of an LOF *CYP2C19* allele has been associated with high-residual platelet reactivity on clopidogrel therapy. In a meta-analysis involving 4341 subjects who received a 600-mg loading dose of clopidogrel, there was a significant high-residual platelet reactivity that seemed to reflect a gene-dose effect in carriers of *CYP2C19*\*2 genotype when compared with noncarriers.<sup>6</sup> In a collaborative meta-analysis that primarily focused on patients who underwent PCI, involving 9685 study participants receiving clopidogrel (91.3% with PCI, 54.5% with acute coronary syndromes), carriers of 1 (hazard ratio, 1.55) or 2 (hazard ratio, 1.76) *CYP2C19* LOF alleles had a significantly increased risk of the composite end point of cardiovascular death, myocardial infarction, or stroke. Pertinent to the case described, there was also an increased risk of stent thrombosis for heterozygotes (hazard ratio, 2.67) and for homozygotes (hazard ratio, 3.97).<sup>7</sup> A subsequent meta-analysis by Holmes et al<sup>6</sup> evaluating 32 studies of 42016



**Figure 2.** Coronary thrombus material extracted from the right coronary artery after thrombectomy.

patients, a treatment-only analysis revealed that carriers of 1 or 2 *CYP2C19* LOF alleles were at a higher risk for cardiovascular events (relative risk, 1.18; absolute risk increase of 8–12 events per 1000 individuals). When this analysis was restricted to studies with  $\geq 200$  events, the relative risk of increased events was not significant. A limitation of this meta-analysis was the inclusion of a large number of patients whom were being treated for reasons other than stenting (eg, atrial fibrillation) in whom the effect of the *CYP2C19* LOF alleles to prevent major adverse cardiovascular events in clopidogrel-treated patients has not been as pronounced. A more recent meta-analysis by Sorich et al<sup>8</sup> demonstrated that the effects of the *CYP2C19* genotype were greatest in patients who undergo PCI. Therefore, there seems to be equipoise in the cardiovascular community whether *CYP2C19*\*2 and \*3 allele carriers are at increased risk for adverse events when treated with clopidogrel especially after PCI.

The *CYP2C19*\*17 allele has been shown, in some studies, to lead to an enhanced response to clopidogrel (via platelet function testing) and a higher rate of bleeding events.<sup>5,9</sup> However, other studies have not demonstrated increased platelet inhibition or altered clinical outcomes with the *CYP2C19*\*17 allele.<sup>10</sup> An individual with the diplotype *CYP2C19* \*2/\*17 is predicted to be a *CYP2C19* intermediate metabolizer; however, data on the effect of this genotype are not consistent, resulting in a provisional status for this classification.<sup>1</sup> The Pharmacogenomics of Anti-Platelet Intervention (PAPI) Study found that the *CYP2C19*\*2 and *CYP2C19*\*17 variants were in linkage disequilibrium, and this finding could account for the negligible effect of \*17 on clopidogrel active metabolite levels and the clopidogrel induced inhibition of platelet aggregation.<sup>11</sup> In our laboratory, \*2 and \*17 variants have been observed to be occurring in *trans* (on opposite chromosomes) in 99.4% of the cases. However, ethnicity of this sample set was unknown; therefore, the frequency of \*2 and \*17 variants occurring in *cis* (on the same chromosome) may be higher in some populations. These findings could potentially explain the variable results observed with the \*17 phenotype.<sup>12</sup>

### Alternatives to Clopidogrel

Newer antiplatelet drugs, such as prasugrel and ticagrelor, have shown benefits over clopidogrel in patients with acute coronary syndrome but also increase the risk of major bleeding.<sup>13</sup> Common genetic variation in *CYP2C19* does not seem to affect prasugrel or ticagrelor pharmacokinetics, its effect on platelet aggregation, or clinical outcomes. Therefore, these therapies may be useful alternatives to clopidogrel in the carriers of *CYP2C19* LOF genetic variants.<sup>14,15</sup> Ticagrelor has to be dosed twice daily and causes dyspnea in 15% of patients,<sup>16</sup> an adverse event that is responsible for 55% of drug withdrawal.<sup>17</sup> Prasugrel cannot be administered to patients with a history of stroke or transient ischemic attacks and is not recommended for patients  $\geq 75$  years of age.<sup>18</sup> Current American College of Cardiology (ACC)/American Heart Association (AHA) guidelines for the use of antiplatelet therapy in acute coronary syndromes (2014), ST-elevation myocardial infarction (2013) and PCI (2011) continue to recommend the use of clopidogrel as a class I, level of evidence B drug.<sup>19–21</sup> Reflective of these guidelines, clopidogrel remains the most widely prescribed antiplatelet drug both in the United States and Canada.<sup>22,23</sup>

## Recommendations for *CYP2C19* Genotyping With Clopidogrel Use

The Food and Drug Administration have issued a black box warning advising practitioners to consider alternative treatment in patients identified as *CYP2C19* poor metabolizers and note that these patients can be identified by genotyping.<sup>24</sup> However, routine clinical use of genotyping to identify *CYP2C19* LOF alleles in patients treated with clopidogrel is not recommended in the latest guidelines published by the ACC, AHA, and Society for Cardiovascular Angiography and Interventions (SCAI).<sup>19–21,25</sup> Clopidogrel treatment failure can be multifactorial and recognition of noncompliance, drug–drug interactions, and relevant comorbidities is critical when addressing treatment success. With these factors in mind, genotyping for *CYP2C19* variant alleles is not recommended by the ACC, AHA, and SCAI because of lack of prospective data from a randomized clinical trial that could adjust for these confounding factors in a systematic way. The 2011 ACC/AHA/SCAI PCI guidelines have recommended (as class IIb) that *CYP2C19* genetic testing may be considered in patients at high risk for poor clinical outcomes.<sup>20</sup> To address the gap in clinical evidence, 2 large, prospective randomized clinical trials, TAILOR-PCI<sup>26</sup> and POPular Genetics study,<sup>27</sup> of genotype-directed antiplatelet therapy compared with routine care are underway.

In contrast to ACC/AHA guidelines, the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend that patients with acute coronary syndrome who undergo PCI and are identified to have 1 or 2 *CYP2C19* LOF alleles are considered to be intermediate and poor metabolizers, respectively, and should be treated with alternative antiplatelet therapy, such as prasugrel or ticagrelor.<sup>1</sup> The strength of the recommendation was considered moderate for heterozygotes or intermediate metabolizers and strong for homozygotes or poor metabolizers. Patients identified to be *CYP2C19* ultrarapid metabolizers, and extensive metabolizers should receive the recommended dose of clopidogrel as per CPIC guidelines. On the basis of these recommendations, several large medical centers have begun providing *CYP2C19* genotyping information preemptively in the medical record to help guide antiplatelet drug use in the post-PCI setting.<sup>28</sup>

## Clinical Case Discussion

In our case, genotyping before PCI was not performed consistent with ACC/AHA guidelines. If genotyping information had been made available before PCI, as per CPIC guidelines, this patient would have been treated with clopidogrel. The pharmacogenetic result did not explain the cause of stent thrombosis. It is entirely possible that the Luminex assay did not detect all variants that could affect *CYP2C19* activity. For example, sequencing the entire *CYP2C19* gene may identify rare LOF variants that affect *CYP2C19* enzymatic activity if a rare LOF allele was not identified with genotyping the patient could have erroneously been classified as having the *CYP2C19*\*1 haplotype. There are also factors other than *CYP2C19* LOF alleles that predispose patients to stent thrombosis, such as variability of drug absorption and drug–drug interactions. Furthermore, propensity for

stent thrombosis is dependent on a myriad of patient comorbidities, including smoking status, diabetes mellitus, chronic kidney disease, type of coronary lesions, and stent-related factors none of which were relevant to our patient.<sup>29</sup> In addition to highlighting the complexity of the use of *CYP2C19* genotyping in clinical management of patients with acute coronary syndrome, our case further supports the lack of evidence supporting the role of *CYP2C19*\*17 as a GOF allele and highlights the importance of performing clinical trials to demonstrate the use of basing therapeutic decisions on pharmacogenetic information.

## Disclosures

None.

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