Clopidogrel Pharmacogenetics
Promising Steps Towards Patient Care?

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Clopidogrel is a pro-drug that requires oxidation to its active metabolite, 2-oxoclopidogrel, by CYP3A4 and other CYP enzymes. This active thiol metabolite inhibits adenosine diphosphate (ADP)-induced platelet aggregation by blocking the platelet P2Y$_{12}$ receptor (Figure), resulting in $\approx 50\%$ reduction in ADP-mediated platelet aggregation. Clopidogrel is standard of care in many patients undergoing percutaneous coronary intervention (PCI) and those experiencing acute coronary syndromes. However, it has been suggested that response to clopidogrel varies widely with nonresponse rates ranging from 4% to 30% at 24 hours.\(^1\,2\) Suggested mechanisms for this variability have included under-dosing, drug interactions with CYP3A4 substrates and inhibitors,\(^3\) and intrinsic interindividual differences resulting from genetic polymorphisms in the pathways of clopidogrel pharmacokinetics and pharmacodynamics.

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In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Angiolillo and colleagues report on the influence of CYP3A4 genotype on interpatient variability in clopidogrel responsiveness.\(^4\) The authors find that an intrinsic single nucleotide polymorphism (SNP) in the CYP3A4 gene, IVS10+12G>A (also called CYP3A4*1G), influences platelet reactivity ex vivo as measured by glycoprotein IIb/IIIa receptor activation in response to clopidogrel in a group of patients with stable coronary disease on established clopidogrel and aspirin therapy. Additionally, they replicated these findings in a group of clopidogrel naïve patients undergoing elective coronary stent placement treated with a 300 mg loading dose of the drug. Ex vivo platelet aggregation profiles in response to clopidogrel did not differ by CYP3A4 genotype.

This study represents the first pharmacogenetic study (i.e., genotype–response association) to focus on candidate genes involved in clopidogrel pharmacokinetics. Other investigators have reported on the influence of phenotypic variability in CYP3A4 activity (measured by erythromycin breath test) on clopidogrel-induced inhibition of platelet aggregation, supporting the notion that genetic influences on metabolism may be important.\(^5\) Clopidogrel pharmacogenetics studies to date have evaluated pharmacodynamic candidate genes including the P2Y$_{12}$ and P2Y$_{1}$ receptors, the glycoprotein Ia and IIa receptor subunit genes, and the protease-activated receptor-1 (PAR-1).\(^5\,7\,10\) These studies have yielded primarily neutral findings\(^5\,7\,10\) with the following exceptions. First, studies evaluating the platelet glycoprotein (GP) Ia gene (ITGA2) have found that among patients on dual therapy with aspirin and clopidogrel, carriers of the 807 T variant allele have increased platelet aggregation compared with 807 C/C individuals.\(^5\,9\) Additionally, although studies evaluating the association of P2Y12 polymorphisms and clopidogrel-induced platelet aggregation have been neutral, Ziegler et al evaluated the effect of two exonic SNPs in P2Y12, 34C>T and 53G>T, on the occurrence of recurrent neurological events in patients with peripheral arterial disease with significant results.\(^12\) They found that in patients treated with clopidogrel, 34T carriers had a 4-fold increased risk of ischemic stroke and/or carotid revascularization compared with 34 C/C individuals.\(^12\) Ex vivo platelet aggregation or reactivity was not measured. The relative contribution of CYP3A4 genotype was not evaluated in these studies.

The cytochrome P450 3A (CYP3A) family of proteins metabolizes half of currently available drugs approved by the Food and Drug Administration. Therefore, polymorphisms in this gene family could play an important role in variability in medication response. However, CYP3A4 is unique from many other polymorphic drug metabolizing enzymes (such as CYP2D6 and CYP2C9) in that, until recently,\(^13\) no null allele had been identified to cause lack of protein formation. The allele that has been identified resulting in nonfunctional protein, CYP3A4*20, is extremely rare.\(^13\) Additionally, expression of CYP3A4 displays a unimodal distribution, with many rare variants contributing to small changes in protein function. With the exception of CYP3A4*1B, no CYP3A4 variant has been consistently associated with a clinical phenotype or with altered CYP3A4 expression and even the CYP3A4*1B allele has not been consistently associated with altered expression.\(^14\) In addition to the IVS10+12G>A SNP (CYP3A4*1G), Angiolillo et al also evaluated the IVS7+258A>G and IVS7+894C>T SNPs and did not find any of them to influence response to clopidogrel. They did not evaluate CYP3A4*1B or CYP3A4*3 because of the infrequent occurrence in their patient population.

Although not evaluated in the present study, CYP3A5 should be investigated as a high priority candidate gene in clopidogrel response. Unlike CYP3A4, CYP3A5 does have polymorphisms resulting in dramatic functional consequences. And, although CYP3A5 protein is present in a
greater proportion of African Americans (≈55%), it is still present in ≈25% of Whites, and perhaps even more frequently among Spanish-Europeans. Interestingly, in the present study, the IVS10+12G>A SNP was in linkage disequilibrium (LD) with CYP3A4*1B, which has been reported to be in strong LD with CYP3A5*1 (which results in increased protein expression). Furthermore, according to HapMap data in the CEPH Whites, IVS10+12G>A and CYP3A5*1 are in LD with a D’ of 0.86, which could be a mechanistic contributor to the increased clopidogrel efficacy seen in CYP3A4 IVS10+12G>A variant carriers.

The only paper (published in abstract form) evaluating CYP3A5 genotype and clopidogrel response to date focused on platelet aggregation, which Angiolillo and colleagues have suggested may not be the optimal phenotype in evaluating responsiveness to clopidogrel.

Several key issues regarding so-called “clopidogrel resistance” must be resolved to determine how large of an impact the findings from the present study will have on patient care. First, the relationship between variability in platelet responsiveness to clopidogrel and thrombotic events has only been demonstrated in a small observational study of 60 patients with ST-elevation myocardial infarction undergoing PCI. Therefore, the clinical relevance of clopidogrel resistance is still a matter of debate. Second, the cut points used in this analysis for considering clopidogrel non-response or low response have been used widely in the literature, but are not based on any data supporting their relation to clinical events and likely vary depending on the clinical indication for clopidogrel. Third, the optimal measure of response to clopidogrel remains to be determined. It remains unclear whether the measurement of platelet aggregation or platelet reactivity is most reflective of clopidogrel response and the assays used to measure these responses as well as the timing of measurement can result in varying response rates. Last, new PDY12 antagonists are in development which may be associated with lower non-response rates. If these agents are approved, they may provide more effective therapeutic alternatives to clopidogrel. Whether the same pharmacogenetic associations seen with clopidogrel will hold true with these agents is not known. If the non-response rates to these new agents are indeed on the order of 10-fold lower than those of clopidogrel as initially reported, clinically important pharmacogenetic associations may be overcome by this improved efficacy.

An interesting area of opportunity for clopidogrel pharmacogenetics involves the recently published CHARISMA trial, in which dual therapy with aspirin and clopidogrel was not beneficial in a group of high risk patients with vascular disease or multiple risk factors. Furthermore, the dual therapy was associated with a “concerning trend” toward an increased risk of bleeding. It is likely that subgroups of patients could be identified using pharmacogenetics to identify those patients most likely to derive benefit from dual therapy, without putting the larger population of patients at risk of severe adverse events. As an important step toward this end, Angiolillo and colleagues provide a provocative
report of an association with an intronic SNP in the CYP3A4 gene and platelet activity in response to clopidogrel. Future studies evaluating whether this polymorphism and those in CYP3A5 predict adverse clinical outcomes in clopidogrel-treated individuals, as well as those determining the functional basis for this association are eagerly awaited.

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