Published in final edited form as:

Catheter Cardiovasc Interv. 2018 June ; 91(7): 1308–1317. doi:10.1002/ccd.27453.

Aspirin and clopidogrel high on-treatment platelet reactivity and genetic predictors in peripheral arterial disease

Khung-Keong Yeo, MBBS^{1,2}, Ehrin J. Armstrong, MD, MSc^{1,3}, Javier E. López, MD⁴, Debbie C. Chen, MD⁵, Gregory G. Westin, MD, MAS⁶, Chin-Shang Li, PhD⁷, David Anderson, MD⁸, Amy Hua⁴, Anil Singapuri⁴, Ezra A. Amsterdam, MD⁴, Nipavan Chiamvimonvat, MD^{4,9}, and John R. Laird, MD¹

¹Division of Cardiovascular Medicine and the Vascular Center, University of California, Davis, CA

²Department of Cardiology, National Heart Centre Singapore, Singapore

³Division of Cardiology, VA Eastern Colorado Healthcare System, CO

⁴Division of Cardiovascular Medicine, University of California, Davis, CA

⁵Department of Medicine, Massachusetts General Hospital, Boston, MA

⁶Division of Vascular and Endovascular Surgery, NYU Langone Medical Center, New York, NY

⁷Division of Biostatistics, Department of Public Health Sciences, University of California, Davis, CA

⁸Department of Internal Medicine, University of California, San Francisco, CA

⁹Department of Veterans Affairs, Northern California Health Care System, Mather, CA

Abstract

Objectives—Our aims were to examine the prevalence and genetic predictors of aspirin and clopidogrel high on-treatment platelet reactivity (HoTPR), and associated adverse cardiovascular outcomes in patients with peripheral arterial disease (PAD).

Background—The association of aspirin and clopidogrel HoTPR with outcomes in PAD remains unclear.

Methods—This is a prospective cohort study of patients with angiographically documented PAD involving carotid and lower extremity arteries. Aspirin and clopidogrel HoTPR (using the VerifyNow Assay) and associated genetic predictors were compared to clinical outcomes. The primary end-point was a composite of major adverse cardiovascular events: all-cause mortality,

Address for correspondence: John R. Laird, MD, UC Davis Medical Center, 4860 Y Street, Suite 3400, Sacramento, CA 95817, Telephone: (916) 734-2028, Fax: (916) 734-2030, jrlaird@ucdavis.edu.

Disclosures:

Dr Yeo: Medtronic: research funding, Abbott Vascular: proctor, speaker; Boston Scientific: consultant, Amgen and Menarini: honorarium

Dr Armstrong: Consultant/Scientific Advisory Board Member for Abbott Vascular, Boston Scientific, Cardiovascular Systems, Medtronic, Spectranetics

Dr Laird: Consultant/Scientific Advisory Board Member for Abbott Vascular, Bard Peripheral Vascular, Boston Scientific, Medtronic.

Dr Amsterdam: Astra Zeneca, advisory board; Relypsa, consultant

myocardial infarction, stroke, target vessel revascularization (TVR) and limb-loss in patients who underwent extremity intervention.

Results—The study was stopped prematurely due to slow patient enrolment. Of 195 patients enrolled, the primary analysis was performed in 154 patients taking both drugs. Aspirin HoTPR was present in 31 (20%) and clopidogrel HoTPR in 76 (49%) patients. There was a trend towards more primary composite outcome events with PRU ≥ 235 , (52% freedom-from-event rate vs. 70% for PRU < 235 ; $p=0.09$). TVR was higher in those with PRU ≥ 235 (20 vs. 6%, unadjusted $p=0.02$). There was no association between aspirin HoTPR and combined outcomes. Single nucleotide polymorphisms in serum paraoxonase/arylesterase 1 (*PON1*) gene was associated with aspirin HoTPR ($p=0.005$) while SNP in phospholipase A2, group III (*PLA2G3*) gene was associated with clopidogrel HoTPR ($p=0.002$).

Conclusion—Clopidogrel HoTPR was significantly associated with TVR, while aspirin HoTPR was not associated with adverse clinical outcomes in patients with PAD.

Keywords

Antiplatelet therapy; peripheral arterial disease; genetics

Introduction

Peripheral arterial disease (PAD) is a major complication of atherosclerosis and is an important aspect of cardiovascular disease. Class 1 recommendation from the American Heart Association and American College of Cardiology management guidelines for PAD patients include the use of antiplatelet agents such as aspirin, with clopidogrel as a reasonable alternative(1–3).

However, there is wide variability in patients' responses to aspirin and clopidogrel and other thienopyridines, resulting in the concept of aspirin and clopidogrel "high on-treatment platelet reactivity" (HoTPR) or "non-responsiveness" or "resistance"(4,5). Aspirin and clopidogrel HoTPR has been correlated with adverse clinical cardiovascular outcomes in coronary artery disease patients(5–8), although this factor has been less well examined in patients with PAD. Multiple genetic polymorphisms have been associated with aspirin and clopidogrel HoTPR. However, these have largely been in coronary artery disease literature(9–12). In the PAD arena, Karnabatidis et al. showed that clopidogrel HoTPR prevalence to be as high as 51% in a cohort of PAD patients. From the same group, Spiliopoulos showed in the PRECLOP study that clopidogrel HoTPR was the only significant predictor of adverse clinical events in a prospective cohort of 100 patients undergoing infrainguinal angioplasty or stenting. In the PRECLOP study, the 1-year cumulative event rate was as high as 52% and 84% in the third and fourth quartiles of clopidogrel HoTPR. Despite these findings, significant gaps of knowledge remain for patients with PAD. Accordingly, the aims of this study are to determine the prevalence of aspirin and clopidogrel HoTPR in a cohort of treated patients with PAD and determine their association with candidate genetic markers, as well as associated adverse cardiovascular outcomes.

Methods

This is a prospective observational cohort study of patients enrolled between August 2010 and September 2012, with angiographically documented PAD involving carotid and lower extremity arteries. Patients may have been treated surgically or endovascularly at the discretion of the primary physician. The University of California Davis institutional review board approved the study. Informed consent was obtained from each patient. The study is registered with ClinicalTrials.gov; with identification number NCT03174990. Inclusion criteria consisted of male and female patients older than 18 years of age undergoing diagnostic angiography (carotid and lower extremity) or therapeutic intervention (surgical or endovascular) for PAD. Exclusion criteria included (1) Patients who were unable to take aspirin AND clopidogrel for any reason; (2) Platelet count < 100K; Hematocrit < 30% or > 52% (limitations of the VerifyNow assay); (3) Patients taking warfarin, ticlopidine, prasugrel, or ticagrelor were excluded from the study. Patients were treated according to operator preference but DAPT was required for a minimum of 30 days if a percutaneous intervention was performed. Continued DAPT beyond 1 month was at the discretion of the operator. The inclusion criteria did not necessitate that patients had to be on DAPT prior to enrolment. We had enrolled patients if they required angiographic evaluation for PAD. However, in some patients, if intervention was not performed or if surgery was performed instead, they would not be on DAPT. The protocol did not further consider the role of any revascularization in its analysis.

Whole blood samples were obtained from each patient via standard venipuncture. Aspirin and clopidogrel HoTPR was tested using the VerifyNow Assay (Accriva Diagnostics, San Diego, CA, USA). For the clopidogrel HoTPR test, the subject had to be on clopidogrel for at least 5 days. If the subject was naïve to clopidogrel, the test was performed 5-7 hours after a loading dose of 300 – 600 mg. Patients would be loaded with aspirin 300mg if they were not already on aspirin. Blood testing for HoTPR for both drugs would be done at the time of clopidogrel HoTPR testing. Blood samples may be obtained any time if a patient was on chronic aspirin and clopidogrel treatment. Aspirin HoTPR was defined as patients with aspirin reaction units (ARU) ≤ 550(13). Clopidogrel HoTPR was defined as patients with P2Y12 reaction units (PRU) ≤ 235(6). The rest of the blood was frozen and retained for genetic analysis.

All patients and investigators were blinded to the test results. Major adverse cardiovascular events (stroke, myocardial and all-cause mortality), limb loss and target vessel revascularization (TVR) at six months and one year were collected using chart review, clinic visits and telephone interviews. Note that TVR only applies if the vessel had been revascularized. The primary end-point was a composite of major adverse cardiovascular events at 1 year, including all-cause mortality, myocardial infarction, stroke, TVR and limb loss in patients who underwent extremity intervention.

Single Nucleotide Polymorphisms (SNP) analyses

Genomic DNA was extracted from whole blood specimens using Gentra Systems PUREGENE DNA Purification Kit (Qiagen). Single nucleotide polymorphisms (SNP) were correlated to measures of aspirin and clopidogrel HoTPR as well as clinical outcomes.

Genotype analysis was performed using the High Throughput Single Nucleotide Polymorphisms analysis (Illumina Golden Gate assay) as well as the DNA sequencing service at the Core Facility of the University of California Genome center.

Candidate gene selection

Candidate gene approach was taken in our SNP analyses based on the known mechanisms of action as well as the absorption and metabolism of aspirin and clopidogrel. Aspirin acts by irreversibly inhibiting the cyclo-oxygenase-1 (COX-1) enzyme, thereby decreasing the production of thromboxane A2. Aspirin also modifies the enzymatic activity of COX-2.

Based on the previously identified genetic polymorphisms influencing the effect of aspirin on platelets, we have included the following polymorphisms in our study: (1) polymorphisms of the COX-1 gene; (2) glycoprotein IIb/IIIa receptor polymorphisms (eg. PIA1 and PIA2 alleles(14–16)); (3) platelet collagen receptors (17), and the GPVI receptor gene (18); (4) ADP receptor P2Y1-12 (19), and (5) Factor XIII (20,21). We have also included phospholipase A2 as well as other enzymes responsible for arachidonic acid metabolisms including cytochrome P450, lipoxygenase and hydrolase.

In addition, previous studies have identified polymorphisms of genes involved in clopidogrel absorption (*ABCB1*), metabolisms (*CYP3A5* and *CYP2C19*) (22), and biologic activity (*P2RY1214* and *ITGB311*)(23,24). Moreover, one recent study has identified *PON1* as a major determinant of clopidogrel efficacy(25). These genes were selected for our candidate SNP analyses.

Sample Size Calculations

Based on studies of high risk patients (including patients with PAD) such as described in the CHARISMA trial, the event rate for aspirin or thienopyridine responsive patients is approximately 7% over 1 year(28). In our study, we included the additional outcome of TVR given the nature of the patients included. We estimated a sample size of 400 patients over 2-years. Assuming a prevalence of either aspirin or thienopyridine HoTPR at 25%(29), a MACE event rate of 20% in the resistant group and 10% in the responsive group, we would have 75% power to detect a clinically important difference at the significance level of 0.05 (95% confidence level) at 1 year follow up. However, due to slow enrolment, the study was terminated upon reaching 195 patients.

Statistical analyses

The two-sided t-test or Wilcoxon rank-sum test as appropriate was used to compare a numerical demographical or clinical characteristic variable between the two groups. The Chi-square test or Fisher's exact test as appropriate was used to compare a categorical demographical or clinical characteristic variable between the two groups. The Kaplan-Meier method was used to estimate an event-free survival curve in a group, and the log-rank test was used to compare the survival distributions of the two groups(30). A multivariable logistic regression was used to study the association between single nucleotide polymorphisms (SNP) in patients with aspirin or clopidogrel high on-treatment platelet reactivity. Genotype frequencies were tested for Hardy–Weinberg equilibrium using the chi-

square test. Genomic analyses were performed using Statistical Analysis Software version 9.4 (SAS Inc. Cary, NC, USA). Clinical analyses were performed using STATA (StataCorp, College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

Results

One-hundred and ninety-five patients were enrolled between August 2010 and September 2012. Although our target enrolment target was 400 patients, due to slow recruitment rates, we were only able to recruit 195 patients. This was largely affected by patients who were anemic and could not be tested by the VerifyNow system. The study was stopped prematurely as a result of slow patient enrolment.

Of the 195 patients, 154 were taking both aspirin and clopidogrel post-procedure. Table 1 shows the clinical characteristics of the cohort prescribed DAPT and the entire cohort of 195 patients. Among the 190 patients prescribed aspirin, 32 (17%) had aspirin HoTPR, as defined by baseline ARU ≥ 550 . Among the 159 patients prescribed clopidogrel, 78 (49%) had clopidogrel HoTPR, as defined by PRU ≥ 235 .

The primary analysis was performed in the 154 patients taking both aspirin and clopidogrel (figure 1). In this cohort, 40% presented with claudication, 27% with critical limb ischemia, and 32% with severe or symptomatic carotid artery stenosis requiring carotid artery angiography and/or stenting. Among these 154 patients, 6 had diagnostic procedures only and the other 148 were intervened upon (96%). Aspirin HoTPR was present in 31 (20%) and clopidogrel HoTPR in 76 (49%) of patients. Table 2 shows the clinical characteristics of patients with and without clopidogrel HoTPR. Table 3 shows the clinical characteristics of patients with and without aspirin HoTPR. Tables 4 and 5 show the rates of occurrence of the combined primary combined endpoint and its individual components at 1 year for patients with clopidogrel and aspirin HoTPR, respectively. Using a combined endpoint at 1 year of myocardial infarction, death, stroke, amputation, or TVR, there was a trend for more events with PRU ≥ 235 , (52% freedom from event rate for clopidogrel HoTPR vs. 70% for no clopidogrel HoTPR p=0.09) (Figure 2). Importantly, TVR was significantly higher in those with PRU ≥ 235 (20 vs. 6%, p=0.02). There was no significant association between aspirin HoTPR and combined outcomes (Figure 3); although there was a trend towards higher rates of stroke and TVR in patients with ARU ≥ 550 (7 vs. 1%, p=0.09; 23 vs. 11%, p=0.08, respectively).

SNP in serum paraoxonase/arylesterase 1 (*PON1*) gene (both heterozygous and homozygous) was associated with aspirin HoTPR (p=0.005) while SNP in phospholipase A2, group III (*PLA2G3*) gene was associated with clopidogrel HoTPR (p=0.002). No other genotypic variations were found for aspirin or clopidogrel HoTPR. Genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) using the chi-square test. All of the reported genotype distributions were in HWE (p values > 0.10).

Discussion

Our study demonstrated that aspirin HoTPR was present in 17% to 20% of patients with PAD while clopidogrel HoTPR was present in approximately 50%. Second, it also showed

that there was a statistical trend for clopidogrel HoTPR to be associated with a combined endpoint of myocardial infarction, death, stroke, amputation, or TVR while there was no significant association between aspirin HoTPR and combined outcomes. Importantly, TVR was significantly higher in those with PRU ≥ 235 . Third, we demonstrate that SNP in serum paraoxonase/arylesterase 1 (*PON1*) gene (both heterozygous and homozygous) was associated with aspirin HoTPR ($p=0.005$) while SNP in phospholipase A2, group III (*PLA2G3*) gene was associated with clopidogrel HoTPR ($p=0.002$).

Significant implications of aspirin and clopidogrel high on-treatment platelet reactivity

In a 2-year follow-up study of 326 stable cardiovascular patients receiving aspirin, Gum et al. showed that aspirin HoTPR was associated with a 4.1-fold excess adjusted hazard of serious vascular events (HR, 4.1; 95% CI, 1.4–12.1)(5). Clopidogrel HoTPR was similarly associated with adverse outcomes. In a study involving 380 patients, Price et al. showed that in patients with drug eluting stents, HoTPR to clopidogrel was associated with significantly higher rates of CV death (2.8 vs. 0%, $P = 0.04$) and stent thrombosis (4.6 vs. 0%, $P = 0.004$) (6). The prevalence of aspirin HoTPR in PAD patients is less clear. Saunders et al. showed in a study of 80 patients that between 5% and 27.5% were poor responders to aspirin when tested over 6- to 12-month follow-up(31). Karnabatidis and colleagues reported in a recent study of 145 patients with PAD, also using the VerifyNow test, that the prevalence of aspirin HoTPR was 20.7%. HoTPR to clopidogrel was 50.8% while 12.5% of patients were non-responsive to both aspirin and clopidogrel(32). The same group extended their investigations and published the PRECLOP study in 2013(33). In this study cohort of 100 patients, Spiliopoulous et al. showed that PRU was a prognostic indicator for the primary combined endpoint of 1-year cumulative clinical events rate (composite endpoint of death, bleeding, major amputation, or clinically driven target vessel re-intervention). Interestingly, the study showed that the optimal cutoff value for the composite endpoint was PRU ≥ 234 . Our findings showed surprisingly similar prevalence rates for clopidogrel and aspirin HoTPR of 50% and 20% respectively. These findings provide independent and corroborative evidence on the likely true prevalence of antiplatelet HoTPR in patients with PAD.

Mueller et al. examined 100 patients who underwent peripheral arterial balloon angioplasty for intermittent claudication, and was able to associate clinical outcome with aspirin HoTPR (34). In the 18-month follow-up period, the risk for arterial re-occlusion in these aspirin-resistant men was 87% higher than in the aspirin-responsive group ($p=0.009$). Unlike the study by Spiliopoulous and Mueller, we were unable to demonstrate any significant association between aspirin or clopidogrel HoTPR and adverse outcomes, although there was a trend towards higher rates of the primary combined endpoint with clopidogrel HoTPR. This may be related to the fact that we included patients with different types of PAD. Conceivably, the selection of only patients who had infrainguinal intervention in the PRECLOP study allowed for greater accrual of events to allow differentiation between those with and without HoTPR. Comparatively, our study cohort included patients with carotid disease, iliac disease, infrainguinal disease and patient were treated endovascularly or surgically. Patients with carotid artery treatment and iliac disease have historically lower rates of target vessel failure compared to infrainguinal disease, thereby accounting for the lower event rates in our study. The mechanism of action arising from clopidogrel HoTPR

resulting in higher rates of TVR is postulated to be related to vascular thrombosis. This may occur in surgically or endovascularly treated patients. However, the data in this study is insufficient to demonstrate this.

Rationale for the selection of candidate SNPs

Aspirin acts by irreversibly inhibiting the cyclo-oxygenase-1 (COX-1) enzyme, thereby decreasing the production of thromboxane A₂. Aspirin also modifies the enzymatic activity of COX-2. Previous studies have suggested that aspirin HoTPR can occur due to polymorphisms in COX1, COX 2, and glycoprotein IIb/IIIa genes. It can also occur if polymorphisms result in the up-regulation of other pathways leading to platelet activation. These include activation by agonists such as ADP, collagen and thrombin. Previously identified genetic polymorphisms influencing the effect of aspirin on platelets include polymorphisms that affect: (1) thromboxane A₂ production resulting from polymorphisms of the COX-1 gene; (2) glycoprotein IIb/IIIa receptor polymorphisms (eg. PIA1 and PIA2 alleles(14–16)); (3) platelet collagen receptors (17), and the GPV1 receptor gene (18); (4) ADP receptor P2Y₁-12 (19), and (5) Factor XIII (20,21). Since aspirin blocks one of the key pathways in arachidonic acid metabolism, we have also included phospholipase A₂ critical for the release of arachidonic acid as well as other enzymes responsible for arachidonic acid metabolisms including cytochrome P450, lipoxygenase and hydrolase.

In addition, previous studies have identified polymorphisms of genes involved in clopidogrel absorption (*ABCB1*), metabolisms (*CYP3A5* and *CYP2C19*) (22), and biologic activity (*P2RY1214* and *ITGB311*)(23,24). Moreover, one recent study has identified *PON1* as a major determinant of clopidogrel efficacy(25). *PON1* gene is located on the long arm of chromosome 7 and encodes for an enzyme hydrolase(26). PON1 is also an anti-atherosclerotic component of high-density lipoprotein and has been shown to be protective against the development of atherosclerosis(27). These genes were selected for our candidate SNP analyses.

Aspirin high on-treatment platelet reactivity

Our analyses identify SNP in serum paraoxonase/arylesterase 1 (*PON1*) gene (both heterozygous and homozygous) to be associated with aspirin HoTPR (p=0.005). *PON1* gene is located on the long arm of chromosome 7 and encodes for an enzyme hydrolase(26). PON1 is also an anti-atherosclerotic component of high-density lipoprotein and has been shown to be protective against the development of atherosclerosis(27). The activity of PON1 is critically affected by genetic polymorphisms(35). Moreover, one recent study has identified *PON1* as a major determinant of clopidogrel efficacy (25). Our data suggesting an association between polymorphisms in *PON1* gene and aspirin HoTPR may directly stem from the variation in the activity of PON1 as a result of genetic polymorphisms. However, the exact mechanisms of PON1 and aspirin HoTPR require further investigations.

Clopidogrel high on-treatment platelet reactivity

Different pathways including those affecting drug absorption, activation, metabolism and biologic activity may modulate Clopidogrel HoTPR. A recent study by Simon et al. showed that the variant allele of *ABCB1* (with TT at nucleotide 3435) had an increased risk of

cardiovascular events in a cohort of patients who present with acute coronary syndrome(23). The same authors also found that patients with any two *CYP2C19* loss-of-function alleles had higher rates of adverse cardiovascular events, especially if they had undergone percutaneous coronary intervention. In our present study, we have identified SNP in phospholipase A2, group III (*PLA2G3*) gene to be associated with clopidogrel HoTPR ($p=0.002$). *PLA2G3* is an extracellular form of phospholipase A2 and has been shown to promote inflammation by catalyzing the release of arachidonic acids(36). Arachidonic acids are further metabolized to form several downstream inflammatory and thrombogenic metabolites. An increase in the level of secreted PLA2 has been shown to be associated with an increase in the incidence of coronary artery disease and acute coronary syndrome(36). Our findings linking genetic polymorphisms and *PLA2G3* gene further support critical roles of arachidonic acid metabolisms in clopidogrel HoTPR.

Limitations

One possible limitation of this study is the use of the VerifyNow assay. However, the test has been shown to correlate well with the ‘gold standard’ of light transmittance aggregometry for aspirin(37,38) or clopidogrel HoTPR (39,40). Most importantly, the VerifyNow assay has been correlated in a number of recent studies to clinical outcomes(6,8,33,41). It has been shown that the clopidogrel efficacy after a loading dose may not represent exactly the effect when the steady-state phase of therapy has been achieved, therefore, it would have been ideal if the timing of testing clopidogrel responsiveness was homogenous.(42,43) However, this was not possible to perform for logistical reasons.

Our study needs to be interpreted in the context of the premature termination of the study due to slow patient enrolment. This resulted in the relatively small sample size limiting our ability to show a statistically significant difference in outcomes between those with and without HoTPR. Although the study initially planned for more patients, slow enrolment made this difficult. The small sample size is especially relevant in terms of the genetic analysis of genotypes that demonstrate association with aspirin and/or clopidogrel HoTPR. Despite the sample size, we were able to demonstrate that clopidogrel HoTPR was associated with TVR. Lastly, the study population included diverse PAD patients including those with carotid artery disease and lower extremity disease; and included those treated endovascularly or surgically. The difference in the rates of major adverse events in these diverse cohorts, as well as the generally low rates of target vessel revascularization in the carotid and iliac arteries (as compared to infrainguinal arteries), all affect the rates of the primary study outcome. Nonetheless, this cohort of patients represents a real-world sampling of PAD. Surprisingly, our prevalence rates of aspirin, clopidogrel and combined HoTPR were very similar to that shown by Karnabatidis et al.

Conclusions

This study shows that aspirin and clopidogrel HoTPR is common. Clopidogrel HoTPR was significantly associated with the specific endpoint of TVR, while aspirin HoTPR was not associated with the defined clinical outcomes. SNP in serum paraoxonase/arylesterase 1 (*PON1*) gene (both heterozygous and homozygous) was associated with aspirin HoTPR

($p=0.005$) while SNP in phospholipase A2, group III (*PLA2G3*) gene was associated with clopidogrel HoTPR ($p=0.002$). The implication of aspirin and clopidogrel HoTPR in patients with PAD and how they can be treated will need to be examined in further studies.

Acknowledgments

Supported by American Heart Association Clinical Research Award (to KKY), NIH (R01 HL085727, R01 HL137228, and R01 HL085844 to NC), VA Merit Review Grant I01 BX000576 and I01CX001490 (to NC), Harold Amos Medical Faculty Development award from the Robert Wood Johnson Foundation and CTSC KL2 scholar supported by the National Center for Advancing Translational Sciences; NIH UL1 TR000002 (to JEL). NC is the holder of the Roger Tatarian Endowed Professor in Cardiovascular Medicine. The project described was supported by the National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), through grant #UL1 TR000002 and grant number #UL1 TR001860. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

1. Rooke TW, Hirsch AT, Misra S, Sidawy AN, Beckman JA, Findeiss LK, Golzarian J, Gornik HL, Halperin JL, Jaff MR, et al. 2011 ACCF/AHA Focused Update of the Guideline for the Management of Patients With Peripheral Artery Disease (updating the 2005 guideline): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2011; 58(19):2020–45. [PubMed: 21963765]
2. Anderson JL, Halperin JL, Albert NM, Bozkurt B, Brindis RG, Curtis LH, DeMets D, Guyton RA, Hochman JS, Kovacs RJ, et al. Management of patients with peripheral artery disease (compilation of 2005 and 2011 ACCF/AHA guideline recommendations): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013; 127(13):1425–43. [PubMed: 23457117]
3. Gerhard-Herman MD, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, Fleisher LA, Fowkes FG, Hamburg NM, Kinlay S, et al. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol*. 2017; 69(11):e71–e126. [PubMed: 27851992]
4. Grottemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder. A pilot-study including 180 post-stroke patients. *Thromb Res*. 1993; 71(5): 397–403. [PubMed: 8236166]
5. Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol*. 2003; 41(6):961–5. [PubMed: 12651041]
6. Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, Ernst A, Sawhney NS, Schatz RA, Teirstein PS. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J*. 2008; 29(8):992–1000. [PubMed: 18263931]
7. Chen WH, Cheng X, Lee PY, Ng W, Kwok JY, Tse HF, Lau CP. Aspirin resistance and adverse clinical events in patients with coronary artery disease. *Am J Med*. 2007; 120(7):631–5. [PubMed: 17602938]
8. Patti G, Nusca A, Mangiacapra F, Gatto L, D'Ambrosio A, Di Sciascio G. Point-of-care measurement of clopidogrel responsiveness predicts clinical outcome in patients undergoing percutaneous coronary intervention results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study. *J Am Coll Cardiol*. 2008; 52(14):1128–33. [PubMed: 18804738]
9. Yin T, Miyata T. Pharmacogenomics of clopidogrel: evidence and perspectives. *Thromb Res*. 2011; 128(4):307–16. [PubMed: 21592545]
10. Cambria-Kiely JA, Gandhi PJ. Aspirin resistance and genetic polymorphisms. *J Thromb Thrombolysis*. 2002; 14(1):51–8. [PubMed: 12652150]

11. Cuisset T, Morange PE, Alessi MC. Recent advances in the pharmacogenetics of clopidogrel. *Hum Genet.* 2012; 131(5):653–64. [PubMed: 22207144]
12. Feher G, Feher A, Pusch G, Lupkovics G, Szapary L, Papp E. The genetics of antiplatelet drug resistance. *Clin Genet.* 2009; 75(1):1–18. [PubMed: 19067731]
13. Lee PY, Chen WH, Ng W, Cheng X, Kwok JY, Tse HF, Lau CP. Low-dose aspirin increases aspirin resistance in patients with coronary artery disease. *Am J Med.* 2005; 118(7):723–7. [PubMed: 15989905]
14. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K. PIA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. *Lancet.* 1997; 349(9049):385–8. [PubMed: 9033464]
15. Szczeklik A, Sanak M, Undas A. Platelet glycoprotein IIIa pl(a) polymorphism and effects of aspirin on thrombin generation. *Circulation.* 2001; 103(6):E33–4. [PubMed: 11171808]
16. Feng D, Lindpaintner K, Larson MG, O'Donnell CJ, Lipinska I, Sutherland PA, Mittleman M, Muller JE, D'Agostino RB, Levy D, et al. Platelet glycoprotein IIIa Pl(a) polymorphism, fibrinogen, and platelet aggregability: The Framingham Heart Study. *Circulation.* 2001; 104(2):140–4. [PubMed: 11447076]
17. Moshfegh K, Willemin WA, Redondo M, Lammle B, Beer JH, Liechti-Gallati S, Meyer BJ. Association of two silent polymorphisms of platelet glycoprotein Ia/IIa receptor with risk of myocardial infarction: a case-control study. *Lancet.* 1999; 353(9150):351–4. [PubMed: 9950439]
18. Lepantalo A, Mikkelsen J, Resendiz JC, Viiri L, Backman JT, Kankuri E, Karhunen PJ, Lassila R. Polymorphisms of COX-1 and GPVI associate with the antiplatelet effect of aspirin in coronary artery disease patients. *Thromb Haemost.* 2006; 95(2):253–9. [PubMed: 16493486]
19. Jefferson BK, Foster JH, McCarthy JJ, Ginsburg G, Parker A, Kottke-Marchant K, Topol EJ. Aspirin resistance and a single gene. *Am J Cardiol.* 2005; 95(6):805–8. [PubMed: 15757620]
20. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost.* 1998; 79(1):8–13. [PubMed: 9459313]
21. Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance. *Int J Clin Pract.* 2007; 61(5):826–34. [PubMed: 17391325]
22. Taubert D, Bouman HJ, van Werkum JW. Cytochrome P-450 polymorphisms and response to clopidogrel. *N Engl J Med.* 2009; 360(21):2249–50. author reply 2251. [PubMed: 19458375]
23. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrieres J, Danchin N, Becquemont L. Genetic Determinants of Response to Clopidogrel and Cardiovascular Events. *N Engl J Med.* 2008
24. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E, et al. Cytochrome P-450 Polymorphisms and Response to Clopidogrel. *N Engl J Med.* 2008
25. Bouman HJ, Schomig E, van Werkum JW, Velder J, Hackeng CM, Hirschhauser C, Waldmann C, Schmalz HG, ten Berg JM, Taubert D. Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med.* 2011; 17(1):110–6. [PubMed: 21170047]
26. Clendenning JB, Humbert R, Green ED, Wood C, Traver D, Furlong CE. Structural organization of the human PON1 gene. *Genomics.* 1996; 35(3):586–9. [PubMed: 8812495]
27. Costa LG, Cole TB, Jarvik GP, Furlong CE. Functional genomic of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. *Annu Rev Med.* 2003; 54:371–92. [PubMed: 12525679]
28. Bhatt DL, Topol EJ. Clopidogrel added to aspirin versus aspirin alone in secondary prevention and high-risk primary prevention: rationale and design of the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial. *Am Heart J.* 2004; 148(2):263–8. [PubMed: 15308995]
29. Hankey GJ, Eikelboom JW. Aspirin resistance. *Lancet.* 2006; 367(9510):606–17. [PubMed: 16488805]
30. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Amer Statist Assn.* 1958; 53(282):457–481.

31. Saunders J, Nambi V, Kimball KT, Virani SS, Morrisett JD, Lumsden AB, Ballantyne CM, Dong JF, Investigators E. Variability and persistence of aspirin response in lower extremity peripheral arterial disease patients. *J Vasc Surg.* 2011; 53(3):668–75. [PubMed: 21227624]
32. Karnabatidis D, Spiliopoulos S, Pastromas G, Kitrou P, Christeas N, Katsanos K, Siablis D. Prevalence of nonresponsiveness to aspirin in patients with symptomatic peripheral arterial disease using true point of care testing. *Cardiovasc Intervent Radiol.* 2014; 37(3):631–8. [PubMed: 23903787]
33. Spiliopoulos S, Pastromas G, Katsanos K, Kitrou P, Karnabatidis D, Siablis D. Platelet responsiveness to clopidogrel treatment after peripheral endovascular procedures: the PRECLOP study: clinical impact and optimal cutoff value of on-treatment high platelet reactivity. *J Am Coll Cardiol.* 2013; 61(24):2428–34. [PubMed: 23602777]
34. Mueller MR, Salat A, Stangl P, Murabito M, Pulaki S, Boehm D, Koppensteiner R, Ergun E, Mittlboeck M, Schreiner W, et al. Variable platelet response to low-dose ASA and the risk of limb deterioration in patients submitted to peripheral arterial angioplasty. *Thromb Haemost.* 1997; 78(3):1003–7. [PubMed: 9308744]
35. Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. *Gen Pharmacol.* 1998; 31(3):329–36. [PubMed: 9703197]
36. Mallat Z, Lambeau G, Tedgui A. Lipoprotein-associated and secreted phospholipases A(2) in cardiovascular disease: roles as biological effectors and biomarkers. *Circulation.* 2010; 122(21):2183–200. [PubMed: 21098459]
37. Paniccia R, Antonucci E, Gori AM, Marcucci R, Poli S, Romano E, Valente S, Giglioli C, Fedi S, Gensini GF, et al. Comparison of different methods to evaluate the effect of aspirin on platelet function in high-risk patients with ischemic heart disease receiving dual antiplatelet treatment. *Am J Clin Pathol.* 2007; 128(1):143–9. [PubMed: 17580282]
38. Frelinger AL, Li Y, Linden MD, Tarnow I, Barnard MR, Fox ML, Michelson AD. Aspirin “resistance”: role of pre-existent platelet reactivity and correlation between tests. *J Thromb Haemost.* 2008
39. Jakubowski JA, Payne CD, Li YG, Brandt JT, Small DS, Farid NA, Salazar DE, Winters KJ. The use of the VerifyNow P2Y12 point-of-care device to monitor platelet function across a range of P2Y12 inhibition levels following prasugrel and clopidogrel administration. *Thromb Haemost.* 2008; 99(2):409–15. [PubMed: 18278193]
40. Paniccia R, Antonucci E, Gori AM, Marcucci R, Giglioli C, Antonucci D, Gensini GF, Abbate R, Prisco D. Different methodologies for evaluating the effect of clopidogrel on platelet function in high-risk coronary artery disease patients. *J Thromb Haemost.* 2007; 5(9):1839–47. [PubMed: 17723123]
41. Marcucci R, Gori AM, Paniccia R, Giusti B, Valente S, Giglioli C, Buonomici P, Antonucci D, Abbate R, Gensini GF. Cardiovascular Death and Nonfatal Myocardial Infarction in Acute Coronary Syndrome Patients Receiving Coronary Stenting Are Predicted by Residual Platelet Reactivity to ADP Detected by a Point-of-Care Assay. A 12-Month Follow-Up. *Circulation.* 2008
42. Savcic M, Hauert J, Bachmann F, Wyld PJ, Geudelin B, Cariou R. Clopidogrel loading dose regimens: kinetic profile of pharmacodynamic response in healthy subjects. *Semin Thromb Hemost.* 1999; 25(Suppl 2):15–9. [PubMed: 10440417]
43. Thebault JJ, Kieffer G, Cariou R. Single-dose pharmacodynamics of clopidogrel. *Semin Thromb Hemost.* 1999; 25(Suppl 2):3–8.

Figure 1.
CONSORT diagram showing the flow of patients.

Figure 2.

Kaplan-Meier curve showing freedom from the primary combined endpoint of MACE, amputation, or TVR, showing trend for more events with PRU ≥ 235 (52% freedom from event rate for clopidogrel HoTPR vs. 70% for no clopidogrel HoTPR, $p=0.09$).

Figure 3.
Kaplan-Meier curve showing no significant association between aspirin HoTPR and the primary combined endpoint.

Table 1

Demographics and clinical characteristics of patients on dual antiplatelet therapy (DAPT) and overall cohort

Variable	DAPT Patients (N=154)	Overall Cohort (N = 195)
Age, years	68 ± 11	63.5 ± 21
Male (%)	96 (62%)	121 (62%)
Race/Ethnicity (%)		
Caucasian	129 (84%)	166 (85%)
Hispanic	9 (6%)	11 (6%)
African American	10 (6%)	12 (6%)
Asian	2 (1%)	4 (2%)
Body mass index, kg/m ²	27.1 ± 6.1	27.5 ± 6.0
Tobacco use		
Never	26 (17%)	33 (17%)
Former	94 (61%)	116 (60%)
Current	33 (22%)	45 (23%)
Congestive heart failure (%)	29 (19%)	36 (19%)
Diabetes mellitus (%)	63 (41%)	76 (39%)
Glomerular filtration rate, ml/min	73 ± 41	75.4 ± 40.0
Hypertension (%)	134 (87%)	169 (87%)
Coronary artery disease (%)	87 (56%)	105 (54%)
History of myocardial infarction (%)	24 (16%)	29 (14%)
Ejection fraction	50 ± 18	52 ± 17%
History of stroke/ transient ischemic attack (%)	39 (25%)	50 (26%)
Chronic obstructive lung disease (%)	24 (16%)	29 (15%)
History of abdominal aortic aneurysm (%)	9 (6%)	13 (7%)
History of gastrointestinal bleed	4 (3%)	5 (2%)
History of prior major amputation (%)	8 (5%)	12 (6%)
ACE inhibitor or Angiotensin receptor blocker (%)	90 (58%)	115 (59%)
Beta blocker (%)	88 (57%)	114 (58%)
Statin (%)	117 (76%)	146 (75%)
Aspirin Post-Procedure	154 (100%)	190 (97%)
Clopidogrel Post-Procedure	154 (100%)	159 (81%)
Presentation		
Claudication	61 (40%)	75 (38%)
Critical Limb Ischemia	41 (27%)	54 (28%)
Carotid Artery Stenosis	49 (32%)	61 (42%)
Acute Limb Ischemia	2 (1%)	3 (2%)
Renovascular Hypertension	1 (1%)	2 (1%)
Ankle Brachial Index (ABI)	0.56 ± 0.25	0.53 ± 0.27
Platelet count (×10 ⁹ /L)	220 ± 75	217 ± 72

ACE: angiotensin converting enzyme inhibitor

Table 2

Characteristics of patients with and without clopidogrel high on-treatment platelet reactivity in the cohort of patients on dual antiplatelet therapy

Variable	Clopidogrel Non-responsiveness (N = 76)	No Clopidogrel non-responsiveness (N=78)	P value
Age, years	66.8 ± 13	69.7 ± 9	0.1
Male (%)	43 (57)	53 (68)	0.1
Race/Ethnicity (%)			0.3
Caucasian	62 (82)	67 (86)	
Hispanic	4 (5)	5 (6)	
African American	7 (9)	3 (4)	
Asian	2 (3)	1 (1)	
Body mass index, kg/m ²	27.4 ± 7.3	26.7 ± 4.6	0.5
Tobacco use			0.6
Never	11 (14)	15 (20)	
Former	49 (63)	45 (60)	
Current	18 (23)	15 (20)	
Congestive heart failure (%)	18 (25)	11 (14)	0.1
Diabetes mellitus (%)	36 (47)	27 (35)	0.1
Glomerular filtration rate, ml/min	69 ± 44	77 ± 39	0.3
Hypertension (%)	66 (87)	68 (87)	0.9
Coronary artery disease (%)	47 (62)	40 (51)	0.2
History of myocardial infarction (%)	14 (18)	10 (13)	0.3
Ejection fraction	47 ± 18	54 ± 17	0.3
History of stroke/ transient ischemic attack (%)	15 (20)	24 (31)	0.1
Chronic obstructive lung disease (%)	10 (14)	14 (18)	0.5
History of abdominal aortic aneurysm (%)	3 (4)	6 (8)	0.4
History of gastrointestinal bleed	3 (4)	1 (1)	0.3
History of prior major amputation (%)	2 (3)	6 (8)	0.4
ACE inhibitor or Angiotensin receptor blocker (%)	43 (57)	47 (60)	0.6
Beta blocker (%)	43 (57)	45 (58)	0.9
Statin (%)	57 (75)	60 (77)	0.8
Presentation			0.3
Claudication	32 (42)	29 (37)	
Critical Limb Ischemia	23 (30)	18 (23)	
Carotid Artery Stenosis	19 (25)	30 (39)	
Acute Limb Ischemia	2 (3)	0 (0)	
Renovascular Hypertension	0 (0)	1 (1)	
Ankle Brachial Index (ABI)	0.58 ± 0.3	0.54 ± 0.2	0.5
Platelet count (×10 ⁹ /L)	223 ± 65	218 ± 84	0.6

ACE: angiotensin converting enzyme inhibitor

Table 3

Characteristics of patients with and without aspirin high on-treatment platelet reactivity in the cohort of patients on dual antiplatelet therapy

Variable	Aspirin Non-responsiveness (N = 31)	No Aspirin non-responsiveness (N= 123)	P value
Age, years	68.6 ± 9.4	68.1 ± 11.8	0.8
Male (%)	20 (65)	76 (62)	0.8
Race/Ethnicity (%)			0.9
Caucasian	27 (87)	102 (83)	
Hispanic	2 (6)	7 (6)	
African American	2 (6)	8 (7)	
Asian	0	2 (2)	
Body mass index, kg/m ²	28.0 ± 4.6	26.9 ± 6.4	0.4
Tobacco use	2		0.8
Never	21 (17)	5 (16)	
Former	76 (62)	18 (58)	
Current	25 (20)	8 (26)	
Congestive heart failure (%)	3 (10)	26 (21)	0.2
Diabetes mellitus (%)	14 (45)	49 (40)	0.6
Glomerular filtration rate, ml/min	92 ± 54	68 ± 36	0.008
Hypertension (%)	25 (81)	109 (89)	0.2
Coronary artery disease (%)	14 (45)	73 (59)	0.2
History of myocardial infarction (%)	3 (10)	21 (17)	0.3
Ejection fraction, %	45 ± 14	50 ± 18	0.7
History of stroke/ transient ischemic attack (%)	8 (26)	31 (25)	0.9
Chronic obstructive lung disease (%)	3 (10)	21 (17)	0.3
History of abdominal aortic aneurysm (%)	2 (7)	7 (6)	0.9
History of gastrointestinal bleed	2 (7)	2 (2)	0.1
History of prior major amputation (%)	3 (10)	5 (4)	0.2
ACE inhibitor or Angiotensin receptor blocker (%)	21 (68)	69 (56)	0.2
Beta blocker (%)	19 (61)	69 (56)	0.6
Statin (%)	21 (68)	96 (78)	0.2
Presentation			0.7
Claudication	11 (35)	50 (41)	
Critical Limb Ischemia	10 (32)	31 (25)	
Carotid Artery Stenosis	10 (32)	39 (32)	
Acute Limb Ischemia	0	2 (2)	
Renovascular Hypertension	0	1 (1)	
Ankle Brachial Index (ABI)	0.54 ± 0.28	0.57 ± 0.25	0.7
Platelet count (×10 ⁹ /L)	222 ± 75	216 ± 73	0.7

ACE: angiotensin converting enzyme inhibitor

Table 4

Event rates at one year for patients with and without clopidogrel non-responsiveness in the cohort of patients on dual antiplatelet therapy

Outcome	PRU <235	PRU ≥235	P value
Primary endpoint *	30%	48%	0.09
Death	10%	9%	0.7
Myocardial infarction	4%	10%	0.3
Stroke	2%	3%	0.6
Major amputation	8%	9%	0.5
TVR	6%	20%	0.02

Event rates are based on Kaplan Meier estimates at one year.

* Defined as the composite of death, myocardial infarction, stroke, major amputation, or TVR (TVR: Target vessel revascularization)

PRU: Plavix reaction units

Table 5

Event rates at one year for patients with and without aspirin resistance

Outcome	ARU <550	ARU ≥550	P value
Primary endpoint *	38%	46%	0.3
Death	8%	9%	0.5
Myocardial infarction	5%	8%	0.7
Stroke	1%	7%	0.09
Major amputation	11%	0%	0.1
TVR	11%	23%	0.08

Event rates are based on Kaplan-Meier estimates at one year.

* Defined as the composite of death, myocardial infarction, stroke, major amputation, or TVR