Pak Heart J

# CLOPIDOGREL RESISTANCE AND ITS GENETICS

**Kashif-ur-Rehman[1], Muhammad Akram Tariq[2],Tanveer Akhtar[3]**

[1,3] Department of Zoology, University of the Punjab, Lahore, Pakistan.
[2] Department of Biomedical Sciences, King Edward Medical University, Lahore, Pakistan.

***Address for Correspondence:***

**Professor Dr. Tanveer Akhtar**

Address: Department of Zoology, University of the Punjab, Lahore (Quaid-i-Azam Campus) Pakistan 54590.

Email: dr_tanveerakhtar@yahoo.com

Date Received: June 18,2013
Date Revised: August 07, 2013
Date Accepted: September 07, 2013

**Contribution**
All the authors contributed significantly to the research that resulted in the submitted manuscript.

**All authors declare no conflict of interest.**

## ABSTRACT

Clopidogrel is an antiplatelet drug that is administered in the form of prodrug that has to be converted to its active metabolite by hepatic cytochrome P450 (CYP) isoenzymes in the liver. There exists individual response variability of platelets inhibition by clopidogrel that is an indication that genetic factors are involved in clopidogrel responsiveness. Combining aspirin and clopidogrel is considered as gold standard therapy for patients who develop acute coronary syndromes (ACS) or are undergoing percutaneous coronary intervention (PCI). However, despite the administration of dual antiplatelet therapy, some patients face recurrent cardiovascular ischemic events, the most catastrophic is stent thrombosis. It is well established that the antiplatelet response to clopidogrel varies widely among patients. Patients who display little attenuation of platelet reactivity under clopidogrel therapy are recognized as low- or non-responders, or clopidogrel-resistant. Different alleles of cytochrome P450 gene and a number of other genes are involved in the pharmacokinetics and pharmacodynamics of clopidogrel. Herein we discussed the mode of action of clopidogrel, mechanism of clopidogrel resistance and relation of genetic polymorphisms of CYP and other genes to clopidogrel resistance in patients using clopidogrel.

**Key Words:** Clopidogrel Resistance, Cytochrome P450 (CYP), ATP2B2, TIAM2.

## INTRODUCTION

Drug resistance is a phenomenon that has received serious attention in recent years in everyday medical practice. This can also be called as low responsiveness or 'non-responsiveness' as patients respond partially to medical treatment or no response at all. Clopidogrel is an antiplatelet drug that is administered in the form of a prodrug to cardiac patients with acute coronary syndrome (ACS) and patients undergoing percutaneous coronary intervention (PCI). Clopidogrel has to be converted to its active metabolite (2-oxo-clopidogrel) by hepatic cytochrome P450 (CYP) isoenzymes in the liver. Clopidogrel functions by inhibiting ADP-induced platelet activation and aggregation by blocking the P2Y12 receptor selectively and irreversibly. [1] It has been reported that clopidogrel non-responsiveness varies between 5% and 44% among different populations. Both the clopidogrel and aspirin are recommended in combination because both therapies inhibit the aggregation of platelets through different ways.[2] As a monotherapy clopidogrel does not effectively inhibit platelet aggregations so it is preferred to use it with aspirin that provides additive advantages in the reduction of atherothrombotic events as compared to either drug alone.[3]

In laboratory terms, the definition of clopidogrel resistance varies depending on the different tests used for quantifying residual platelet reactivity and the selection of cut-off values. More specifically, when light transmittance aggregometry is used, the optimal threshold for defining high residual platelet reactivity is set as a percentage of platelet inhibition lower than 20%, or induced maximal platelet aggregation greater than 50%.[4-6] Although light transmission aggregometry was considered as the gold standard for the evaluation of antiplatelet drug responsiveness until recently yet the assay has drawbacks such as lack of standardization and poor specificity in assessing P2Y12 inhibition. Therefore, new and more specific assays may be more suitable to assess the clopidogrel potency. [7]

## MECHANISM OF ACTION

The clopidogrel is a prodrug belonging to thienopyridine group. It is metabolized by hepatic cytochrome P450 enzyme (CYP) to an active metabolite that targets the platelet P2Y12 receptor

specifically and irreversibly. Only a small proportion of the clopidogrel is metabolized by CYP enzyme. The major amount of clopidogrel is hydrolysed by esterases into its inactive carboxylic acid derivative which is round about 85% of the circulating compounds in the plasma related to clopidogrel. CYP3A4 and CYP3A5 enzymes that are found in human liver, metabolize clopidogrel faster than other human CYP isozymes. This suggests that these enzymes are involved in the in vivo clopidogrel metabolism.[8] CYP enzymes catalyze the oxidation of thiophene ring of clopidogrel into 2-oxo-clopidogrel.[8,9] The 2-oxo intermediate is further oxidized by the CYP. The second step of oxidation results in the opening of thiophene ring forming both a carboxyl and a thiol group.[8] The free thiol group in the active metabolite of clopidogrel inactivates the P2Y12 receptor by forming a disulfide bridge with one or both of two free cysteine residues (Cys17 and Cys270) in the extracellular domain of this receptor and ADP cannot bind to the covalently modified receptor.[8] Although clopidogrel 75mg daily dose inhibits the ADP induced platelet aggregation as early as two hours after the first dose, but it takes about 3-7 days to achieve maximum platelet aggregation inhibition. However,this delayed inhibition can be speeded up with the loading dose of clopidogrel [10], as the administering loading dose 300mg of clopidogrel is helpful in shortening the peak effect time to 12 hours.[11] This peak effect period can further be shortened to 2-3 hours by increasing the loading dose of clopidogrel upto 600mg.[12] However, the increased loading dose of clopidogrel from 600 to 900mg is unable to suppress the ADP induced platelet aggregation as the plasma concentration of the active metabolite of clopidogrel does not increase.[13] This is an important information that, even a 600mg loading dose of clopidogrel is unable to achieve the complete inhibition platelet aggregation induced by ADP. The average of the maximum platelet aggregation induced with 5 mol/L ADP in the time period of 4 hours after the administering 600 mg loading dose of clopidogrel is in the range of 50%.[11]

## CLOPIDOGREL RESISTANCE

### a) DEFINITION OF CLOPIDOGREL RESISTANCE

It is the widely accepted fact that there is a significant inter-individual difference in the platelet inhibition after administering clopidogrel. [9,14,15] There seems to be interindividual difference in the anti-inflammatory effect of clopidogrel which has not been established yet.[16,17] Different researchers have given different definitions of drug resistance as the characterization of resistance involves complications. At present clopidogrel resistance could not be defined clearly. Common term 'resistance' is used when a drug becomes unable to achieve its pharmacological effect due to inability of the drug to reach its target or due to the alterations in the target site, and the term 'failure of therapy' is used for those patients who have recurrent events despite use of therapy.[18-20] A single agent with its antiplatelet effect cannot completely prevent and treat ischaemic events due to the complex pathophysiology of ischaemic heart disease which involves thrombosis, inflammation, vascular biology and haemodynamics etc. A patient can achieve appropriate platelet response with the given therapy but at the same time he can experience recurrent ischaemic events induced by some non-platelet factors.[19] The non-

responsiveness to the Clopidogrel ranges from 5-56% in patients undergoing coronary stenting. [21] This difference is mainly due to the following factors: (a) different methods used to monitor the action of clopidogrel; (b) difference in concentrations of the agonist used to induce platelet aggregation; (c) use of different anticoagulants to preserve the samples; (d) different criterias and cut off values used to measure clopidogrel resistance; and (e) difference in timings in the measurement of platelet reactivity after the use of clopidogrel.

It is worthnoting that poor response to clopidogrel can lead to increase incidence of recurrent adverse cardiovascular events. In case-control studies it has been shown that, patients with stent thrombosis have increased ADP-induced platelet reactivity and incomplete inhibition of P2Y12 receptor despite use of clopidogrel.[22,23] It has been demonstrated in many clinical studies that there is an association between increased risk of cardiovascular events and clopidogrel's non-responsiveness.[24]

### b) MECHANISM OF CLOPIDOGREL RESISTANCE

There are different mechanisms responsible for clopidogrel resistance. One of the most widely studied phenomenon is genetic polymorphisms. Clopidogrel is a prodrug which requires two steps performed by CYP enzymes to convert it into its active metabolites. The genes which encode these enzymes are polymorphic in nature, with specific alleles that are associated with decreased enzyme activity and as a result reduced production of active metabolites. CYP2C19 encodes a series of isozymes which are involved in clopidogrel metabolism and both stages of clopidogrel biotransformation. The frequency of common polymorphisms of CYP2C19 enzyme varies from 30-55% in the population, depending on the genetic and ethnic groups which affect the individual response to clopidogrel both pharmacokinetically and pharmacodynamically.[25-31]

### c) EVALUATION OF CLOPIDOGREL RESPONSE IN LABORATORY

There is lack of standardized laboratory techniques for the measurement of in vivo platelet response to antiplatelet therapy, as the clopidogrel inhibits one of the two ADP receptors specifically. The most common method used for ex-vivo measurement of ADP-induced platelet aggregation is light transmittance aggregometry (LTA). It is considered the gold standard method uptill now.[14] As the antiplatelet drugs including clopidogrel are involved in the reduction of platelet aggregation, so it has been suggested that better measurement of antiplatelet activity with clopidogrel is achieved at the time 6 minutes after stimulation with ADP.[32]

It has been discovered in patients with stents that maximum and final aggregation of platelets in clopidogrel treated patients were equivalent in determining the prevalance of clopidogrel's nonresponsiveness. Other techniques including Flow Cytometry for the expression of activated GP IIb/IIIa receptor and expression of p-selectin after the stimulation of ADP are also helpful in the measurement of nonresponsiveness of clopidogrel. [23] As point of care assays, VerifyNow P2Y12 receptor assay and Whole blood thrombelastography are also being used to measure the responsiveness of clopidogrel by using ADP as agonist.[23,33]

## GENETIC POLYMORPHISMS IN CYP AND OTHER GENES

Inspite of considerable clinical efficacy of clopidogrel therapy there is recurrence of cardiovascular events in patients treated with clopidogrel. One of the possible reasons for occurrance of adverse cardiovascular events in patients treated with clopidogrel therapy is the inter-individual response variability. The proposed mechanisms involved in the variability to clopidogrel therapy include genetic, cellular and clinical causes.[4,34] Currently, a trial has been performed with the name of GWAS i.e a genome-wide association study. In this study 429 homozygous healthy white subjects were selected to monitor ADP-induced platelet aggregation in response to clopidogrel. Further, it was confirmed that impaired inhibitory effect of clopidogrel is strongly associated with CYP2C19*2 variant. Further, it has been revealed that a cluster of 13 SNPs (all of these were in strong linkage disequilibrium with each other) spanning 1.5 megabases on chromosome 10q24 was significantly associated with reduced platelet inhibition by the clopidogrel. This cluster of SNPs is located within the CYP2C18-CYP2C19-CYP2C9-CYP2C8 gene cluster, and it encodes corresponding Cytochrome P450s isozymes which play an important role in drug metabolism and bioactivation of clopidogrel. It was revealed further that the loss-of-function allele CYP2C19*2 has strong association with the original 10q24 signal.[35]

Large focus has been given to the genetic factors with special emphasis on the genetic variants of CYP2C19 isoforms. These CYP isoforms are involved in the inter-individual response variability.[27,36] The polymorphisms in the CYP2C19 in response to clopidogrel therapy is thought to be involved in both steps of clopidogrel's hepatic metabolism. The carriers of at least one “poor metabolizer allele” of CYP2C19 (either *2 or *3) have lower levels of active metabolite of clopidogrel and have reduced platelet inhibition.[34] It has been demonstrated that the determination of plasma troponin levels is based on the comparisons of allelic frequencies of variants of CYP2C19 in Australian population.[37] In Korean population, CYP2C19*3 polymorphism has affected clopidogrel resistance significantly in patients with coronary artery disease.

Carriers of CYP2C19*3A allele had a high proportion of clopidogrel resistance.CYP2C19*3 may have an important role in the activity of CYP gene than other reported variants.38 Significant inter-racial differences in the frequency of CYP2C19*3 gene having 9 of 34 alleles have been detected in poor metabolizers from Japan. However, due to the lack of CYP2C19*3A allele in Caucasian population, its role was not completely studied,Table 1.[38]

Pharmacokinetic and pharmacodynamic changes attributed by genetic polymorphisms in CYP2C19 gene in healthy volunteers[27,28] and in patients have been widely investigated (39,40,41). Subjects carrying one or two CYP2C19 loss of function alleles have shown lower plasma concentrations of active metabolites of clopidogrel when compared with subjects without CYP2C19 variant alleles.[28] Similarly, worse clinical outcomes have been observed in patients carrying two loss of function alleles of CYP2C19 for cloidogrel, while patients carrying one loss of function allele for CYP2C19 did not show any risk. [42] However, targeting of individualized therapy can play an important role in improving clinical outcomes after the implantation of stent. There are multiple factors which are still unknown in describing the clinical role of genetic profiling. Genetic polymorphisms of CYP2C19 have shown to reduce the clinical efficacy and metabolism of clopidogrel. Studies are needed to evaluate the clinical benefits of individualized antiplatelet therapy on the basis of genotyping. Patients carrying loss of function alleles CYP2C19*2 and CYP2C19*3 for clopidogrel metabolism have increased risk of thrombosis.[43] There also exists inter-ethnic variability in CYP2C19 gene in response to clopidogrel which ranges from 20-30% in Caucasians, 30-45% among African-Americans and 50-65% in East Asians. Whereas CYP2C19*2 allele is the most frequent loss of function allele i.e 75-85% in Caucasians and East Asians. [44] There exists a correlation between the carriers of reduced function variants for CYP2C19*2 or any of two loss of function alleles (*2,*3,*4 or *5) and higher cardiovascular adverse events rate. [45] Now it has been elaborated that carriers of atleast one reduced function CYP2C19 allele face reduction of the active metabolite of clopidogrel in the plasma up to 32.4% compared to healthy gene carriers. [46] This reduction is especially observed in patients with CYP2C19*2 allele as a best indicator of reduced response to clopidogrel. [35,47,48,49,50] Recent studies have indicated that CYP2C19*3 and *4 alleles may also reduce response to clopidogrel in the same manner as CYP2C19*2 do. [51,52] Genetic polymorphisms in the enzymes other than CYP2C19 may also be associated with clopidogrel's reduced response in patients i.e in carriers of reduced response CYP2C9 and CYP2B6 genes are involved.[28,46] As far as the distribution of CYP alleles responsible for clopidogrel resistance is concerned, there are about 26% Caucasians who are

**Table 1: Distribution of CYP Genetic Polymorphisms in clopidogrel responsive and resistant groups in Korean patients Source: (Lee et al. 2009, Ref: 38)**

| Gene Name | SNP ID | Genotype | NoResistance (n = 275) | Resistance (n=112) | p Value |
|---|---|---|---|---|---|
| CYP2C19*2 | rs4244285 | Codominant (GG,GA, AA) | (155, 93,26) | (55,40,13) | 0.287 |
| | | Dominant (GG,GA/AA) | (155, 119) | (55,53) | 0.361 |
| | | Recessive (GG/GA,AA) | (248, 26) | (95,13) | 0.457 |
| CYP2C19*3 | rs4986893 | Codominant (GG,GA,AA) | (236,37,1) | (80,31,01) | 0.001 |
| | | Dominant (GG,GA/AA) | (236,38) | (80,32) | 0.001 |
| | | Recessive (GG/GA,AA) | (273,1) | (111,01) | 0.497 |
| CYP3A5 | rs776746 | Codominant (GG,GA,AA) | (154,102,12) | (61,41,06) | 0.808 |
| | | Dominant (GG,GA/AA) | (154,114) | (61,47) | 0.908 |
| | | Recessive (GG/GA,AA) | (256,12) | (102,06) | 0.606 |
| CYP3A4 | rs2242480 | Codominant (GG,GA,AA) | (172, 90,13) | (74,32,06) | 0.568 |
| | | Dominant (GG,GA/AA) | (172, 103) | (74,38) | 0.561 |
| | | Recessive (GG/GA,AA) | (262, 13) | (106,06) | 0.798 |

heterozygous for a loss of function allele CYP2C19*2, while 2% are homozygous. This is the most frequent genotype in Asians.[53,54] There is a higher on clopidogrel treatment platelet reactivity in healthy subjects carrying one or two loss of function alleles CYP2C19*2 or CYP2C19*3 as compared to homozygotes carrying CYP2C19*1 wild type allele.[27,28,55]

Genetic polymorphisms in the enzymes other than CYP2C19 may also be associated with clopidogrel's reduced response in patients i.e in carriers of reduced response CYP2C9 and CYP2B6 genes are involved. [28,46] Some genes other than CYP are also involved in clopidogrel resistance. A recent study has identified two new genes involved in biological efficacy of clopidogrel, the first gene is ATP2B2 which is responsible for encoding an ion transport protein involved in the intracellular calcium homeostasis and the second gene is TIAM2 which encodes a protein involved in platelet aggregation.[56]

## CLOPIDOGREL RESISTANCE IN PAKISTAN SCENARIO

There is only one study available regarding the response variability of clopidogrel and aspirin combination therapy on Pakistani population. In this study, it has been shown that dual antiplatelet resistance i.e low response to combination therapy with clopidogrel and aspirin is a common problem in Pakistani cardiac patients with acute coronary syndrome (ACS). There is no significant effect of age and gender on platelet aggregability. The platelet aggregation was measured by using the technique of whole blood aggregometry.[57] The data regarding clopidogrel resistance is scarce in pakistani population and therefore, data of pharmacogenetics of clopidogrel is utmost important for screening resistant mutations against this drug which will help in tailoring the antiplatelet therapy in these patients and to reduce the risk of recurrent stent thrombosis and other adverse cardiovascular events like MI and stroke.

## CONCLUSION

In recent years, the use of clopidogrel has been increased tremendously. This increased use of drug is due to its significant beneficial effects in patients having Acute Coronary Syndrome (ACS) and in patients who undergo Percutaneous Coronary Interventions (PCI). However, the inter-individual response variability in these patients is an emerging problem in recent years. Genetic Polymorphisms in Cytochrome P450 (CYP) gene are responsible for the reduced response to clopidogrel which results in recurrence of thrombosis and other adverse cardiovascular events. So, it is the need of hour to screen these mutations in patients to avoid the abovementioned complications and guide them for the better selection of antiplatelet therapy either alone or in combination with aspirin or some other P2Y12 receptor antagonist. This strategy can mark the beginning of an era of individualized antiplatelet therapy in the near future. In Pakistan scenario, if initiated, this mutation screening for clopidogrel resistance will be the first of its nature.

## REFERENCES


1. Ding Z, Kim S, Dorsam RT, Jin J, Kunapuli SP. Inactivation of the human P2Y12 receptor by thiol reagents requires interaction with both extracellular cysteine residues, Cys17 and Cys270. Blood 2003;101:3908-14.
2. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Sabate M, Barrera-Ramirez C, et al. Identification of low responders to a 300 mg clopidogrel loading dose in patients undergoing coronary stenting. Thromb Res 2005;115:101-8.
3. Antman EM, Hand M, Armstrong PW, Bates ER, Green LA, Halasyamani LK, et al. 2007 focused update of the ACC/AHA 2004 guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines.J Am Coll Cardiol 2008;51:210-47.
4. Angiolillo DJ, Shoemaker SB, Desai B. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes ellitus (OPTIMUS) study. Circulation 2007;115:708-16.
5. Wiviott SD, Trenk D, Frelinger AL, O'Donoghue M, Neumann FJ, Michelson AD, et al. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: the prasugrel in comparison to clopidogrel for inhibition of platelet activation and aggregation-thrombolysis in myocardial infarction 44 trial. Circulation 2007;116:2923-32.
6. Aradi D, Vorobcsuk A, Lenkey Z, Horváth IG, Komócsi A. Low platelet disaggregation predicts poor response to 150 mg clopidogrel in patients with elevated platelet reactivity. Platelets 2010;21:1-10.
7. Bouman HJ, Parlak E, van Werkum JW, Breet NJ, ten Cate H, Hackeng CM, et al. Which platelet function test is suitable to monitor clopidogrel responsiveness? A pharmacokinetic analysis on the active metabolite of clopidogrel. J Thromb Haemost 2010;8:482-8.

8. Clarke TA, Waskell LA. The metabolism of clopidogrel is catalyzed by human cytocrhome P450 3A and is inhibited by atorvatstatin. Drug Metab Dispos 2003;31:53-9.

9. Pereillo JM, Maftouh M, Andrieu A, Uzabiaga MF, Fedeli O, Savi P, et al. Structure and stereochemistry of the active metabolite of clopidogrel. Drug Metab Dispos 2002;30:1288-95.

10. Bates ER, Lau WC, Bleske BE. Loading, pretreatment, and inter-individual variability issues with clopidogrel dosing. Circulation 2005;111:2557-9.

11. Giuliano RP, Braunwald E. The year in non-ST-segment elevation acute coronary syndromes. J Am Coll Cardiol 2005;46:906-19.

12. Hochholzer W, Trenk D, Frundi D, Blanke Giuliano RP, Braunwald E. The year in non-ST-segment elevation acute coronary syndromes. J Am Coll Cardiol 2005;46:906-19.

13. Von Beckerath N, Taubert D, Pogatsa-Murray G, Schomig E, Kastrati A, Schomig A. Absorption, metabolization, and antiplatelet effects of 300, 600, and 900 mg loading doses of clopidogrel. Results of the ISAR-CHOICE (Intracoronary stenting and antithrombotic regimen: choose between 3 high oral doses for immediate clopidogrel effect) trial. Circulation 2005;112:2946-50.

14. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908-13.

15. Matetzky S, Shenkman B, Guetta V, Schechter M, Bienart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004;109:3171-5.

16. Kleiman NS. Platelets, the cardiologist, and coronary artery disease: moving beyond aggregation. J Am Coll Cardiol 2004;43:1989-91.

17. Xiao Z, Theroux P. Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. J Am Coll Cardiol 2004;43:1982-8.

18. Cattaneo M. Aspirin and clopidogrel: efficacy, safety, and the issue of drug resistance. Arterioscler Thromb Vasc Biol 2004;24:1980-7.

19. Wiviott SD, Antman EM. Clopidogrel resistance.Anew chapter in a fast-moving story. Circulation 2004;109:3064-7.

20. Nguyen TA, Diodati JG, Pharand C. Resistance to clopidogrel: a review of the evidence. J Am Coll Cardiol 2005;45:1157-64.

21. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. J Am Coll Cardiol 2005;45:246-51.

22. Barragan P, Bouvier JL, Roquebert PO, Macaluso G, Commeau P, Comet B, et al. Resistance to thienopyridines: clinical detection of coronary stent thrombosis by monitoring of vasodilator-stimulated phosphoprotein phosphorylation. Catheter Cardiovasc Interven 2003;59:295-302.

23. Gurbel PA, Bliden KP, Guyer K, Cho PW, Zaman KA, Kreutz RP. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. J Am Coll Cardiol 2005;46:1820-6.

24. Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events afterdrug-eluting stent implantation. Eur Heart J 2008;29:992-1000.

25. Savi P, Combalbert J, Gaich C, Rouchon MC, Maffrand JP, Berger Y, et al. The antiaggregating activity of clopidogrel is due to a metabolic activation by the hepatic cytochrome P450-1A. Thromb Haemost 1994;72:313-7.

26. Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet 2002;41:913-58.

27. Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood 2006;108:2244-7.

28. Brandt JT, Close SL, Itturia SJ, Payne CD, Farid NA, Ernest CS, et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 2007;5:2429-36.

29. Kim KA, Park PW, Hong SJ, Park JY. The effect of CYP2C19 polymorphism on the pharmacokinetics and pharmacodynamics of clopidogrel: a possible mechanism for clopidogrel resistance. Clin

Pharmacol Ther 2008;84:236-42.

30. Varenhorst C, James S, Erlinge D, Brandt JT, Braun OO, Man M, et al. Genetic variation of CYP2C19 affects both pharmacokinetic and pharmacodynamic responses to clopidogrel but not prasugrel in aspirin treated patients with coronary artery disease. Eur Heart J 2009;30:1744-52.

31. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, et al. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos 2010;38:92-9.

32. Labarthe B, Theroux P, Angioi M, Ghitescu M. Matching the evaluation of the clinical efficacy of clopidogrel to platelet function tests relevant to the biological properties of the drug. J Am Coll Cardiol 2005;46:638-45.

33. Von Beckerath N, Pogatsa-Murray G, Wieczorek A, Sibbing D, Schomig A, Kastrati A. Correlation of a new point-of-care test with conventional optical aggregometry for the assessment of clopidogrel responsiveness. Thromb Haemost 2006;95:910-2.

34. Ferreiro JL, Angiolillo DJ. Clopidogrel response variability: current status and future directions. Throm Haemost 2009;102:7-14.

35. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB. Association of cytochrome P450 2 C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA 2009;302:849–57.

36. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dörrler K, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. Eur Heart J 2009;30:916-22.

37. Dear AE, Deam D, Tanti TT, Byron KB. Proceedings of Haematology Society of Australia Annual Scientific Meeting, October 2009. Kent Town SA: Festival City Conventions; 2009.

38. Lee JM, Park S, Shin DJ, Choi D, Shim CY, Ko YG, et al. Relation of genetic polymorphisms in the cytochrome p450 gene with clopidogrel resistance after drug eluting stent implantation in Koreans. Am J Cardiol 2009;104:46-51.

39. Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, et al. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. Pharmacogenet Genomics 2007;17:1057-64.

40. Fontana P, Senouf D, Mach F. Biological effect of increased maintenance dose of clopidogrel in cardiovascular outpatients and influence of the cytochrome P450 2C19*2 allele on clopidogrel responsiveness. Thromb Res 2008;121:463-8.

41. Trenk D, Hochholzer W, Fromm MF. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol 2008;51:1925-34.

42. Simon T, Verstuyft C, Mary-Krause M, Drouet E, Meneveau N, Steg G, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 2009; 360:363-75.

43. Harmsze AM, van Werkum JW, ten Berg JM, Zwart B, Bourman HJ, Breet NJ, et al. CYP2C19*2 and CYP2C9*3 alleles are associated with stent thrombosis: a case control study. Eur Heart J 2010;31:3046-53.

44. Man M, Farmen M, Dumaual C, Teng C, Moser B, Irie S, et al. Genetic variation in metabolizing enzyme and transporter gene: comprehensive assessment in 3 major East-Asian sub-populations with comparison to Caucasians and Africans. J Clin Pharmacol 2010;50:929-40.

45. Holmes DJ, Dehmer G, Kaul S, Leifer D, O' Gara P, Stein C. ACCF/AHA Clopidogrel clinical alert: approaches to the FDA "boxed warning": a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and

the American Heart Association. Circulation 2010;122:537-57.

46. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms, response to clopidogrel. N Engl J Med 2009;360:354-62.

47. Fontana P, Hulot JS, De Moerloose P, Gaussem P. Influence of CYP2C19 and CYP3A4 gene polymorphisms on clopidogrel responsiveness in healthy subjects. J Thromb Haemost 2007;5:2153-5.

48. Frere C, Cuisset T, Morange PE, Quilici J, Camoin-Jau L, Saut N, et al. Effect of cytochrome P450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. Am J Cardiol 2008;101:1088-93.

49. Geisler T, Zürn C, Paterok M, Göhring-Frischholz K, Bigalke B, Stellos K, et al. Statins do not adversely affect post-interventional residual platelet aggregation and outcomes in patients undergoing coronary stenting treated by dual antiplatelet therapy. Eur Heart J 2008;29:1635-43.

50. Jinnai T, Horiuchi H, Makiyama T, Tazaki J, Tada T, Akao M, et al. Impact of CYP2C19 polymorphisms on the antiplatelet effect of clopidogrel in an actual clinical setting in Japan. Circ J 2009;73:1498-503.

51. Lee SW, Park SW, Kim YH, Yun SC, Park DW, Lee CW, et al. Drug-eluting stenting followed by cilostazol treatment reduces late restenosis in patients with diabetes mellitus the DECLARE-DIABETES trial (A Randomized Comparison of Triple Antiplatelet Therapy with Dual Antiplatelet Therapy After Drug-Eluting Stent Implantation in Diabetic Patients). J Am Coll Cardiol 2008;51:1181-7.

52. Gladding P, Webster M, Zeng I, Farrell H, Stewart J, Ruygrok P. The pharmacogenetics and pahrmacodynamics of clopidogrel response: an analysis from PRINC (Plavix Response in Coronary Intervention) trial. JACC Cardiovasc Interv 2008;1:620-7.

53. Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Blinden K. Reduced function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. J Am Med Assoc 2010;304:1812-30.

54. Jang JS, Cho KI, Jin HY, Seo JS, Yang TH, Kim DK. Meta-analysis of cytochrome P450 2C19 polymorphism and risk of adverse clinical outcomes among coronary artery disease patients of different ethnic groups treated with clopidogrel. Am J Cardiol 2012;110:502-8.

55. Umemura K, Furuta T, Kondo K. The common gene variants of CYP2C19 affect pharmacokinetics and pharmacodynamics in an active metabolite of clopidogrel in healthy subjects. J Thromb Haemost 2008;6:1439-41.

56. Price MJ, Carson AR, Murray SS, Phillips T, Janel L, Tisch R, et al. First pharmacogenomic analysis using whole exome sequencing to identify novel genetic determinants of clopidogrel response variability: results of the Genotype Information and Functional Testing (GIFT) exome study. J Am Coll Cardiol 2012;59:9.

57. Faheem M, Shah I, Ali J, Adil M, Ahmed Z, Hafizulah M. Frequency of dual antiplatelet resistance with coronary artery disease. J Med Sci 2012;20:176-80.