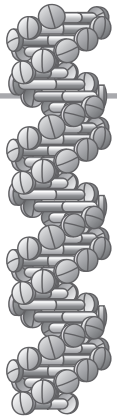

Current status of clopidogrel pharmacogenomics

"...evidence demonstrates that genetic variants, in particular CYP2C19*2 *polymorphism, are independent determinants of residual platelet reactivity and prognosis in high-risk vascular patients on antiplatelet treatment."*

KEYWORDS: acute coronary syndrome ■ antiaggregant ■ bioinformatics ■ clopidogrel ■ genetic testing ■ pharmacogenetics ■ pharmacogenomics ■ predictive algorithms

Antiaggregant therapy with aspirin and clopidogrel has an important role in treatment of patients with acute coronary syndrome (ACS) to reduce the risk of major adverse cardiovascular events (MACE; myocardial infarction, cardiovascular death, stent thrombosis and stroke) [1]. Clopidogrel has a substantial benefit in high-risk patients undergoing percutaneous coronary intervention (PCI) and stent implantation. Despite adequate antiaggregant treatment, MACE occur in these patients. Patients who develop MACE have a high-on-clopidogrel platelet reactivity (HPR), suggesting that inadequate responsiveness to clopidogrel is one of the main causes of thrombotic events [1–3].

Multiple chronic or transient mechanisms are involved in HPR and risk of MACE [4]. Among these mechanisms, genetic polymorphisms play a pivotal role and represent a possible tool for clinicians to improve the management of high-risk vascular patients requiring clopidogrel [4–6].

Since 2006, studies in healthy subjects and in ACS patients undergoing PCI on clopidogrel treatment showed that a *CYP2C19*2* polymorphism is an independent predictor of clopidogrel response and occurrence of MACE [4]. Therefore in 2010, owing to the power of the evidence, the US FDA added a boxed warning to the clopidogrel label to alert clinicians to the impact of the *CYP2C19* genotype on the drug's efficiency, thereby leaving the decision to use genetic testing to the discretion of physicians.

We believe that a clear definition of usefulness of clopidogrel pharmacogenetics is particularly important in a context in which new alternative drugs with different characteristics and costs are available. Indeed, the generic version of clopidogrel is still available, while the patents for the brand name versions of prasugrel and ticagrelor will expire in 2012 and 2018, respectively. We underline that the possibility to choose the most efficient and economic pharmacologic strategy is important for all patients and, in particular, in health systems in which the healthcare expenditure is totally borne by the patient.

The thienopyridine clopidogrel is a prodrug, absorbed at intestinal levels through P-glycoprotein coded for by the *ABCB1* gene, it is mostly hydrolyzed by esterases to an inactive derivative (85%). Only 15% of clopidogrel undergoes CYP450 hepatic metabolism [4]. The active metabolite links to the $P2Y_{12}$ ADP receptor and causes an irreversible blockade of ADP binding [4]. Prasugrel is rapidly hydrolyzed by carboxyesterases to a thiolactone, which is subsequently metabolized to form an active metabolite via one-step CYP450 metabolism, with a lesser contribution from CYP2C19 with respect to clopidogrel [4]. Prasugrel's active metabolite binds irreversibly to the $P2Y_{12}$ receptor. By contrast, ticagrelor does not require hepatic conversion into an active metabolite, and reversibly binds to the ADP receptor [4]. Prasugrel and ticagrelor have been shown to be more potent platelet inhibitors and to have a more favorable benefit-to-risk ratio compared with clopidogrel in high-risk patients. This discrepancy comes at the expense of shifting the balance towards an increased risk of bleeding especially in some patient categories (old age, underweight and prior cerebral events) [7,8]. Moreover, other antiaggregant drugs such as iloprost, cilostazol and cangrelor are available and might be considered in the management of high-risk vascular patients [4].

Several *CYP2C19* variants have been identified and exhibit functional impairment, which determines the poor metabolizer status of subjects. The *CYP2C19*2* polymorphism (rs4244285) is a

Betti Giusti

Author for correspondence: Department of Medical & Surgical Critical Care, University of Florence – Atherothrombotic Diseases Center, Careggi Hospital, Largo Brambilla 3, 50134 Florence, Italy
Tel.: +39 0557949420
Fax: +39 0557949418
betti.giusti@unifi.it

Anna Maria Gori

Rossella Marcucci

Rosanna Abbate

For a full list of affiliations, please see page 1674

Future Medicine part of fsg

G681A nucleotide substitution in intron 4/exon 5 junction, which introduces a splicing defect, resulting in a truncated, nonfunctional protein [4–6]. The *CYP2C19*2* variant allele has a different frequency in Caucasians (15%), Asians (30%) and African–Americans (17%) [4–6]. The *CYP2C19*3* polymorphism (rs4986893) is a G636A nucleotide substitution in exon 4 that creates a premature stop codon, resulting in a truncated, metabolically inactive protein [4–6]. The *CYP2C19*3* polymorphism is less frequent in Asians (5%) and rare in both Caucasians (0.04%) and African–Americans (0.4%) [4–6]. Other rare variants determine the poor metabolizer phenotype, such as *CYP2C19*4* (rs28399504, A-to-G substitution in the initiation codon, resulting in a Met1Val substitution) and *CYP2C19*5* (rs56337013, C1297T substitution in exon 9, resulting in an Arg433Trp substitution) [4].

In the 2007, our group first demonstrated the effect of *CYP2C19*2* polymorphism in modulating platelet function in ACS patients undergoing PCI on clopidogrel [9]. The **2* allele was associated with HPR and furthermore **2*2* homozygous subjects had significantly higher platelet aggregation than **1*2* heterozygotes. Other evidence confirmed that the *CYP2C19*2* polymorphism is associated with HPR in similar and different clinical settings [4].

Most importantly, in 2009, different researchers simultaneously demonstrated that *CYP2C19*2* is a determinant of the occurrence of MACE, and in particular stent thrombosis, in different clinical settings of patients on clopidogrel [4]. By contrast, Paré *et al.* showed that the presence of the *CYP2C19*2* polymorphism in patients with ACS (CURE) or atrial fibrillation (ACTIVE-A) was not associated with an increased risk of MACE [10].

Several meta-analyses of clopidogrel pharmacogenetics are available in the literature with apparently different responses to a similar interrogation [11–14]. These apparent discrepancies are used to support doubts on the usefulness of clopidogrel pharmacogenetics. The first meta-analysis by Sofi *et al.* on data from six prospective studies indicated that the *CYP2C19*2* polymorphism is associated with increased risk of MACE and in particular stent thrombosis [11]. Two larger studies by Hulot *et al.* [12] and the collaborative meta-analyses from Mega *et al.* [13], demonstrated that carriage of even one reduced-function *CYP2C19* allele is associated with a significantly increased risk of MACE and stent thrombosis. Accordingly, with these observations, *CYP2C19* genetic information identifies approximately 30% of the population who may be less likely to be protected from recurrent ischemic events after PCI despite treatment with standard doses of clopidogrel. A further meta-analysis of 32 studies by Holmes *et al.* came to different conclusions [14]. By including all studies in which clopidogrel pharmacogenetics were tested relative to cardiovascular events, independently from the fact that they studied high-risk patients undergoing PCI or lower-risk vascular patients, the authors concluded that the status of *CYP2C19*2* carriers was not clinically relevant for cardiovascular outcomes with the possible exception of stent thrombosis [14].

> *"To choose the 'superior' drug on the basis of large trials in which participants with different risk profiles are considered equal may not be the best strategy."*

The hazard of drawing conclusions to apply in clinical practice by acquiring data from systematic reviews or meta-analyses including and considering similar patients with greatly different risks of cardiovascular events, and therefore different benefit from clopidogrel treatment should be underlined. Indeed, in their study, Holmes *et al.* paid particular attention to include comparator trials of clopidogrel treatment response in which both treatment and comparator group were genotyped (CLARITY-TIMI 28, CURE, ACTIVE-A and CHARISMA) [14]. In these studies, populations consisted of patients that, in large part, have substantial differences in the cardiovascular risk in comparison to studies considered in previous meta-analyses: patients receiving fibrinolytic therapy of whom 50% also had PCI (CLARITY-TIMI 28), with non-ST elevation myocardial infarction of whom 18% had PCI (CURE), with atrial fibrillation (ACTIVE-A) in whom the benefit of clopidogrel in terms of risk reduction was 11%, and with documented cardiovascular disease or risk for cardiovascular disease not showing benefit of clopidogrel over placebo (CHARISMA) [14].

Concerning *CYP2C19* variants, a further polymorphism, *CYP2C19*17* (rs12248560), has been discovered that results in an increased enzyme function due to a -806C/T substitution in the 5′-flanking region of the gene, which causes increased transcription [15]. In patients undergoing PCI, Sibbing *et al.* showed that *CYP2C19*17* carrier status is significantly associated with enhanced response to clopidogrel, and an increased risk of bleeding [15]. This datum

was confirmed by further evidence suggesting the need of its introduction in the diagnostic panel of clopidogrel pharmacogenetic variants [5,6].

Other polymorphisms were studied, but at present clear evidence on their role have not been achieved. Contemporarily, Mega *et al.* [16] in TRITON-TIMI38 and Wallentin *et al.* [17] evaluated the role of the C3435CT *ABCB1* polymorphism on the efficacy of antiaggregant treatments obtaining opposite results on definition of the risk allele. Bouman *et al.* evaluated the clinical relevance of the Q192R polymorphism of *PON1*, an enzyme involved in clopidogrel bioactivation, in patients who underwent stent implantation [18]. QQ192 *PON1* homozygous patients showed a considerably higher risk than RR192 homozygous patients of stent thrombosis, lower PON1 plasma activity, lower plasma concentration of clopidogrel active metabolite and lower platelet inhibition [18]. Cayla *et al.*, evaluating clinical, angiographic and genetic factors, identified *CYP2C19*2*, *CYP2C19*17*, C3435CT *ABCB1* and *PLA1/A2 ITGB3*, but not Q192R *PON1* polymorphisms independently associated with early coronary stent thrombosis [19].

Recently, evidence from prospective studies has accumulated in support of the successful validation and clinical application of the clopidogrel pharmacogenetics in the management of patients undergoing PCI [20,21].

In order to introduce clopidogrel pharmacogenetics into the routine management of ACS patients, the major concerns raised by the scientific community are represented by economic issues and by the immediate availability of genetic test results. Several rapid systems for genetic testing are now commercially available [20–22]. These systems guarantee results in 1–3 h, are point-of-care tests usable that can be used by unskilled personnel, have a cost of approximately €100, which will be amortized by the reduction of social and economic costs of the management of clinical complications in these high-risk patients. A further, non-marginal, issue to be overcome is the concern deriving from the lack of confidence in the management of genetic test results by clinicians. This is a diffuse and broad issue not only for the specific clopidogrel pharmacogenetics, but in general for pharmacogenetics and systems medicine in the present and in the future.

Conclusion & future perspective

The scientific knowledge deriving from genetics, pharmacogenetics, transcriptomics, metabolomics, microbiome, proteomics and imaging is rapidly growing and the major challenge is/will be to translate and integrate this mass of information into consolidated disease knowledge and procedures for the management of patients. Three major issues need to be addressed and improved: the development of adequate bioinformatics approaches; the continuous multidisciplinary training of all the experts involved in the process (biologists, medical specialists, medical geneticist and bioinformatics experts); and the development of diagnosis and treatment paths under the guidance of the system medicine principles.

> *"We believe that for clopidogrel, the definition from a consensus task of algorithms for the evaluation of antiplatelet treatments tailored to individual characteristics of patients ... is urgently needed to identify therapeutic strategies that will provide the best benefit for the single patient in this high-risk clinical setting."*

In conclusion, evidence demonstrates that genetic variants, in particular *CYP2C19*2* polymorphism, are independent determinants of residual platelet reactivity and prognosis in high-risk vascular patients on antiplatelet treatment. To choose the 'superior' drug on the basis of large trials in which participants with different risk profiles are considered equal may not be the best strategy. We believe that for clopidogrel, the definition from a consensus task of algorithms for the evaluation of antiplatelet treatments tailored to individual characteristics of patients (genetic profile, residual platelet reactivity, drug–drug interactions and traditional and procedural risk factors) is urgently needed to identify therapeutic strategies that will provide the best benefit for the single patient in this high-risk clinical setting.

Financial & competing interests disclosure

R Marcucci has received honoraria from Daiichi Sankyo/Eli Lilly and Merck Sharp & Dohme for lectures. R Abbate has received consulting fees from Eli Lilly; lecture fees from Instrumentation Laboratory and Sigma Tau; and research grant funding from Bayer, Boehringer Ingelheim and Pfizer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Bonello L, Tantry US, Marcucci R *et al.* Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. *J. Am. Coll. Cardiol.* 56(12), 919–933 (2010).

2 Sofi F, Marcucci R, Gori AM, Giusti B, Abbate R, Gensini GF. Clopidogrel nonresponsiveness and risk of cardiovascular morbidity: an updated meta-analysis. *Thromb. Haemost.* 103(4), 841–848 (2010).

3 Parodi G, Marcucci R, Valenti R *et al.* High residual platelet reactivity after clopidogrel loading and long-term cardiovascular events among patients with acute coronary syndromes undergoing PCI. *JAMA* 306(11), 1215–1223 (2011).

4 Giusti B, Gori AM, Marcucci R, Saracini C, Vestrini A, Abbate R. Determinants to optimize response to clopidogrel in acute coronary syndrome. *Pharmacogenom. Person. Med.* 3, 33–50 (2010).

5 Krishna V, Diamond GA, Kaul S. Do platelet function testing and genotyping improve outcome in patients treated with antithrombotic agents?: the role of platelet reactivity and genotype testing in the prevention of atherothrombotic cardiovascular events remains unproven. *Circulation* 125(10), 1288–1303 (2012).

6 Gurbel PA, Tantry US. Do platelet function testing and genotyping improve outcome in patients treated with antithrombotic agents?: platelet function testing and genotyping improve outcome in patients treated with antithrombotic agents. *Circulation* 125(10), 1276–1287 (2012).

7 Wiviott SD, Braunwald E, McCabe CH *et al.* Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* 357, 2001–2015 (2007).

8 Wallentin L, Becker RC, Budaj A *et al.* Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* 361, 1045–1057 (2009).

9 Giusti B, Gori AM, Marcucci 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* 17, 1057–1064 (2007).

10 Paré G, Mehta SR, Yusuf S *et al.* Effects of *CYP2C19* genotype on outcomes of clopidogrel treatment. *N. Engl. J. Med.* 363(18), 1704–1714 (2010).

11 Sofi F, Giusti B, Marcucci R, Gori AM, Abbate R, Gensini GF. Cytochrome P4502C19*2 polymorphism and cardiovascular recurrences in patients taking clopidogrel: a meta-analysis. *Pharmacogenomics J.* 11(3), 199–206 (2011).

12 Hulot JS, Collet JP, Silvain J *et al.* Cardiovascular risk in clopidogrel-treated patients according to cytochrome P450 2C19*2 loss-of-function allele or proton pump inhibitor coadministration: a systematic meta-analysis. *J. Am. Coll. Cardiol.* 56(2), 134–143 (2010).

13 Mega JL, Simon T, Collet JP *et al.* Reduced-function *CYP2C19* genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *JAMA* 304(16), 1821–1830 (2010).

14 Holmes MV, Perel P, Shah T, Hingorani AD, Casas JP. *CYP2C19* genotype, clopidogrel metabolism, platelet function, and cardiovascular events: a systematic review and meta-analysis. *JAMA* 306(24), 2704–2714 (2011).

15 Sibbing D, Koch W, Gebhard D *et al.* Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation* 121(4), 512–518 (2010).

16 Mega JL, Close SL, Wiviott SD *et al.* Genetic variants in *ABCB1* and *CYP2C19* and cardiovascular outcomes after treatment with clopidogrel and prasugrel in the TRITON-TIMI 38 trial: a pharmacogenetic analysis. *Lancet* 376(9749), 1312–1319 (2010).

17 Wallentin L, James S, Storey RF *et al.* Effect of *CYP2C19* and *ABCB1* single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 376(9749), 1320–1328 (2010).

18 Bouman HJ, Schömig E, van Werkum JW *et al.* Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat. Med.* 17(1), 110–116 (2011).

19 Cayla G, Hulot JS, O'Connor SA *et al.* Clinical, angiographic, and genetic factors associated with early coronary stent thrombosis. *JAMA* 306(16), 1765–1774 (2011).

20 Roberts JD, Wells GA, Le May MR *et al.* Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. *Lancet* 379(9827), 1705–1711 (2012).

21 Campo G, Parrinello G, Ferraresi P *et al.* Prospective evaluation of on-clopidogrel platelet reactivity over time in patients treated with percutaneous coronary intervention relationship with gene polymorphisms and clinical outcome. *J. Am. Coll. Cardiol.* 57(25), 2474–2483 (2011).

22 Saracini C, Vestrini A, Galora S, Armillis A, Abbate R, Giusti B. Pharmacogenetics of clopidogrel: comparison between a standard and a rapid genetic testing. *Genet. Test. Mol. Biomarkers* 16(6), 500–503 (2012).

Affiliations

- **Betti Giusti**
  Author for correspondence:
  Department of Medical & Surgical Critical Care, University of Florence – Atherothrombotic Diseases Center, Careggi Hospital, Largo Brambilla 3, 50134 Florence, Italy
- **Anna Maria Gori**
  Department of Medical & Surgical Critical Care, University of Florence – Atherothrombotic Diseases Center, Careggi Hospital, Largo Brambilla 3, 50134 Florence, Italy
  and
  Don Carlo Gnocchi Foundation, Scientific Institute, Florence, Italy
- **Rossella Marcucci**
  Department of Medical & Surgical Critical Care, University of Florence – Atherothrombotic Diseases Center, Careggi Hospital, Largo Brambilla 3, 50134 Florence, Italy
- **Rosanna Abbate**
  Department of Medical & Surgical Critical Care, University of Florence – Atherothrombotic Diseases Center, Careggi Hospital, Largo Brambilla 3, 50134 Florence, Italy