Feasibility and implementation of *CYP2C19* genotyping in patients using antiplatelet therapy

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Aim: A tailored antiplatelet strategy based on *CYP2C19* genotype may reduce atherothrombotic and bleeding events. We describe our experience with *CYP2C19* genotyping, using on-site TaqMan or Spartan genotyping or shipment to a central laboratory. **Methodology:** Data from two ongoing projects were used: Popular Risk Score project (non-urgent percutaneous coronary intervention patients) and the Popular Genetics study (ST-segment elevation myocardial infarction patients). For both projects, the time to genotyping result was calculated. **Results:** In the Popular Risk Score project (n = 2556), median time from blood collection to genotyping result was 4:04 h. In the Popular Genetics study (n = 1038), median time from randomization to genotyping result was 2:24 h. **Conclusion:** *CYP2C19* genotyping is feasible in everyday clinical practice, both in the acute and non-acute settings.

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Clopidogrel is the P2Y₁₂ inhibitor of choice in patients after planned percutaneous coronary intervention (PCI). It is also still widely used in patients with acute coronary syndrome (ACS), although the European Society of Cardiology guidelines recommend the more potent P2Y₁₂ inhibitors prasugrel and ticagrelor in those patients [1,2]. The higher efficacy with regard to the prevention of atherothrombotic events when using prasugrel or ticagrelor, however, comes at the cost of an increased bleeding risk and higher drug costs [3,4].

Clopidogrel is subject to a wide interpatient variation in drug response, influenced by genetic factors, clinical factors and drug–drug interactions (i.e., calcium channel blockers, certain proton pump inhibitors such as omeprazole and esomeprazole or St John's Wort) [5–7]. Clopidogrel is a prodrug that needs to be activated by hepatic CYP450 enzymes to become effective [8]. Patients who are carrier of one or more *CYP2C19* loss-of-function alleles have a lower active metabolite level [9–12], higher level of platelet reactivity and an increased risk for recurrent atherothrombotic events, in particular in the patients with the highest thrombotic risk [13–16].

In contrast, ticagrelor does not need biotransformation to become effective, and, although CYP2C19 is involved in prasugrel metabolism as well, the *CYP2C19* genotype does not have a clinically relevant influence on prasugrel efficacy [17,18]. Non-functional *CYP2C19* polymorphisms are no rare phenomena, while for Caucasian patients, the allele frequency of *CYP2C19**2 (rs4244285, 681G>A) is approximately 15% and approximately 0.4% for *CYP2C19**3 (rs4986893, 636G>A). In Asian patients, those numbers are even higher (respectively, 29–35% and 2.4–8.9%) [19].

The finding of a diminished clopidogrel efficacy in patients carrying a *CYP2C19* loss-of-function allele has led to the addition of a black-box warning to the clopidogrel label by the US FDA, who warned physicians that an alternative antiplatelet drug should be considered in *CYP2C19**2 homozygote (poor-metabolizer) patients [20]. Furthermore, the EMA gave a warning for a diminished effect in *CYP2C19* poor-metabolizer patients [21]. The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline as well as the Dutch Pharmacogenetics Working Group guideline state that an alternative antiplatelet agent for clopidogrel should be considered in ACS and PCI patients who are intermediate or poor metabolizer for *CYP2C19* [19,22].

Randomized trials testing personalized treatment strategies based on *CYP2C19* genotype in patients treated with P2Y₁₂ inhibitors are ongoing, such as the Popular Genetics study in patients with ST-segment elevation myocardial infarction (STEMI) undergoing primary PCI [23], and the Tailored Antiplatelet Initiation to Lessen Outcomes Due to Decreased Clopidogrel Response after Percutaneous Coronary Intervention (TAILOR-PCI) study in PCI patients (ClinicalTrials.gov NCT01742117). Those personalized treatment strategies are based on the hypothesis that clopidogrel efficacy is comparable to ticagrelor or prasugrel in the subgroup of normal metabolizer (*1/*1) patients, while the bleeding risk might actually be lower [4,11,16,24]. Therefore, treating *CYP2C19* normal-metabolizer ACS patients with clopidogrel, although not recommended by current non-STEMI and STEMI guidelines, might reduce bleeding risk and drug costs, without an increase in thrombotic events [1,2]. However, to make these personalized medicine strategies a success, *CYP2C19* genotyping has to be feasible in everyday clinical practice.

In this paper, the feasibility of *CYP2C19* genotyping is studied, using the data of two projects in which *CYP2C19* genotyping is used to tailor antiplatelet treatment.

Methods

Popular risk score project

The Popular Risk Score project was a single-center prospective registry that included consecutive patients in whom non-urgent PCI with stent implantation was performed. A risk score was calculated in all patients, consisting of platelet reactivity testing using the VerifyNow P2Y₁₂ assay (Accriva Diagnostics, CA, USA), *CYP2C19**2 and *3 genotyping using the TaqMan StepOnePlus assay, and clinical variables (diabetes, adjoined stent length >30 mm and left ventricular ejection fraction <30%). The risk score and associated platelet function and genetic testing were performed as part of routine clinical care. Patients with a high risk score were treated with prasugrel for 1 year, while patients with a low risk score were treated with clopidogrel for 1 year [25].

Implementation

In all patients planned for PCI, one ethylenediaminetetraacetic acid (EDTA) tube with blood was collected using venapuncture before the procedure to perform *CYP2C19* genotyping. When a blood sample could not be obtained before PCI, a blood sample was collected during PCI from the arterial catheter or after PCI using venapuncture (in a small proportion of patients). The technicians of the pharmacogenetics laboratory collected the samples on a routine basis three-times a day (at 8:00, 10:00 and 14:00 h) and performed genotyping for *CYP2C19**2 and *3. Genotyping results were reported in the hospital electronic patient file. After the PCI, the medical physician responsible for the Cathlab reviewed all results and decided, based on the calculated Popular Risk Score, if the antiplatelet therapy had to be changed.

Validation

*CYP2C19**2 and *3 genotyping was performed by using a real-time PCR technique with TaqMan Genotyping Master Mix and TaqMan Drug Metabolism Genotyping assay performed on the StepOnePlus (ThermoFisher, MA, USA). The assays were validated by the pharmacogenetics laboratory before applying the test in routine patient care. As part of the validation process, selected samples with different genotypes based on the results of the TaqMan assay were also sequenced. Genotypes obtained by both methods were equal.

Popular genetics study

The Popular Genetics study (ClinicalTrials.gov, NCT01761786) is an ongoing international multicenter, open-label, randomized clinical trial consisting of patients with STEMI in whom primary PCI was performed. The rationale and design of this study have been published earlier [23]. In short, patients were randomized to a control group, treated with ticagrelor or prasugrel for 1 year, or an intervention group in which *CYP2C19**2 and *3

genotyping was performed. Normal metabolizer patients (*1/*1) were treated with clopidogrel, while patients carrying one or two loss-of-function alleles (*2 or *3) were treated with prasugrel or ticagrelor. *CYP2C19**17 was not tested.

Different *CYP2C19* genotyping strategies were used in different study sites: on-site testing using the TaqMan StepOnePlus assay, on-site genotyping using the Spartan RX Point-of-Care (POC) device or shipment to a central laboratory using postal or courier service for TaqMan StepOnePlus genotyping. In the central laboratory (which is the same laboratory in which genotyping is performed for the Popular Risk Score project), *CYP2C19* genotyping is routinely performed three-times daily on working days (Monday to Friday) and one time daily on Sunday. No routine testing was performed on Saturday.

Implementation

An EDTA blood sample was collected from all study patients and sent to the central laboratory. Genotyping for *CYP2C19**2 and *3 was performed in patients randomized to the genotyping arm, after written informed consent was obtained. During the course of this study, a Spartan RX genotyping device was made available by the company in seven centers for use in this study. A buccal swab was collected and directly analyzed in the genotyping device in these centers. In one center, the Spartan RX genotyping was performed by laboratory personnel; in the other centers Spartan RX genotyping was performed by a local study investigator or a study nurse. Users were trained by the Spartan company personnel in person or via a video conference session. The device could be placed in the clinical chemistry laboratory, catheterization laboratory or coronary care unit. From six centers without on-site genotyping capability, blood samples were shipped to the study's central laboratory for TaqMan genotyping. Results from central laboratory testing were reported in the local electronic patient file and sent to the referring hospital by email by the coordinating study investigator. In one center, on-site TaqMan genotyping was used. This center was also the central laboratory site. Over time, centers could change their genotyping strategy – that is, when a Spartan RX device came available. All genotyping results were sent to the patient's general practitioner, treating cardiologist and local pharmacy.

Validation

Because central laboratory testing was performed in the same laboratory where the Popular Risk Score genotyping was performed, validation was equal to the method described above. For confirmation purposes, 199 patients tested with the Spartan RX device were also genotyped using TaqMan genotyping on the blood sample sent to the central laboratory.

Statistical analysis

Statistical analyses were performed using SPSS software (v24.0, IBM, USA). For patients in the Popular Risk Score project, the time between blood collection and reporting of the genotyping test result was calculated. For patients in the Popular Genetics study, the time between randomization and reporting of the genotyping test result was calculated. The time between blood collection and test result was not considered the most relevant measure to study the feasibility of the different genotyping methods, because of the design of the study: genotyping could only be performed after Informed Consent was obtained and randomization was performed.

Results

Popular risk score

Between July 2010 and July 2016, 2556 patients were genotyped for the purpose of calculating the Popular Risk Score. Those patients were on average 65 years of age (± 10.7 years) and 74.3% of the patients were male. Distribution of *CYP2C19* genotype is shown in Figure 1A. Median time between blood collection and genotyping result was 4:04 h ([interquartile range (IQR) 2:27, 16:57], range 1:16–96:51 h) (Figure 2). For 49.1% of patients, the *CYP2C19* genotyping result was made available to the treating medical physician within 4 h after PCI, in 71.9% at the same day PCI was performed, in 94.5% within 24 h and in 96.9% within 48 h after PCI. In almost all patients in whom genotyping result took more than 48 h, the blood sample was collected at Friday afternoon, after the last genotyping round. In those patients, genotyping results were available the following Monday morning.

In the Popular Risk Score cohort, 2199 patients (86.0%) received coronary stent implantation. In those patients, data of P2Y₁₂ inhibitor use following the PCI were available in 1449 patients (65.9%). In total, 991 patients (68.4%) had a low risk score and were advised to use clopidogrel. This advice was followed in 988 patients (99.7%);

Figure 1. *CYP2C19* allele frequency. (A) *CYP2C19* allele frequency in the Popular Risk Score project; (B) *CYP2C19* allele frequency in the Popular Genetics study.

Figure 2. Time between blood collection (A) or randomization (B) and *CYP2C19* genotyping results, for each genotyping method. (A) Popular Risk Score project; (B) Popular Genetics study. CL: Central laboratory.

one patient had an allergy for clopidogrel, one patients received coronary artery bypass grafting after which the P2Y₁₂ inhibitor was stopped, and for one patient it was not clear why the risk score advice was not followed. In total, 456 patients (31.5%) were advised to use prasugrel or ticagrelor, which was followed in 405 patients (88.8%); three patients received clopidogrel because of a high bleeding risk, two because of concomitant use of acenocoumarol, two patients had a contraindication for prasugrel (previous stroke) and in 44 patients, it was not clear why the risk score advice was not followed.

Popular Genetics study

All patients included between July 2014 (study initiation) and 8th December 2017 in the Popular Genetics study, and randomized to the genotyping arm, were selected. This selection contained 1283 patients. Because it is an ongoing trial, data were not yet entered in the electronic case record form for a part of the patient cohort. The electronic case record form was complete with data describing the method of genotyping and the time between randomization and genotyping result in 1038 patients (80.9%) from nine study sites. These patients were on average 61.9 years of age (± 11.2 years) and 74.3% of the patients were male. On-site TaqMan genotyping was used by one study site (487 patients), on-site Spartan RX genotyping by seven study sites (411 patients) and shipment to the central laboratory was used by six study sites (140 patients). Distribution of *CYP2C19* genotype is shown

in Figure 1B. The median time between randomization and genotyping result was 2:24 h ([IQR 1:46, 12:53], range 0:30–13:32 h); 2:04 h (1:44, 3:58) for in-hospital TaqMan genotyping, 2:16 h (1:38, 4:29) for Spartan RX genotyping and 52:32 h (28:16, 80:13) for central laboratory testing (Figure 2 & Supplementary Materials). For all patients in the database tested with the Spartan RX device, the test results were inconclusive in 39 patients (8.0%). This was solved by shipment to the central laboratory for TaqMan genotyping (30 patients), or repeated Spartan RX testing (two patients). In seven patients, no further genotyping was performed; in five patients ticagrelor was continued, one patient was switched to clopidogrel because a vitamin K antagonist was started and the discharge medication of the remaining patient was unknown. In one patient, the Spartan RX result did not match the TaqMan result, which was most likely due to contamination of the Spartan RX sample. Repeated testing of the same patient gave results matching the central laboratory result.

Discussion

In this analysis, we evaluated the feasibility of different strategies of *CYP2C19* genotyping, used to tailor antiplatelet therapy in patients admitted with STEMI or undergoing a planned PCI. We conclude that it is feasible to have genotyping results available within 24–48 h in the majority of patients, using TaqMan StepOnePlus genotyping or the Spartan RX POC device. As expected, shipment to a central laboratory takes more time than on-site genotyping.

The use of the Spartan RX POC device has advantages over TaqMan genotyping. First, results can be available within 1 h after collection of a buccal swab. This makes it possible for patients undergoing planned PCI to have genotyping results available before same day discharge. Furthermore, in the acute setting of myocardial infarction antiplatelet therapy might be adjusted shortly after hospital arrival, even during weekend-, evening- or night time hours. Second, the use of a buccal swab is more patient friendly compared with venapuncture for an additional blood sample. Third, although the test must be performed by trained personnel, the POC device can be situated outside the laboratory – in example, in the Cathlab or on the CCU. Another disadvantage for TaqMan genotyping is that laboratory personnel performing the test have to be trained to a higher level and a certified laboratory has to be available.

There are also disadvantages related to POC testing. First, although confirmation studies show a good correlation between Spartan RX and laboratory-based genotyping [26,27], in one patient the Spartan test result did not match with TaqMan genotyping result, and in 8% of patients in our cohort the test result was inconclusive on the first attempt. Although this problem might be solved at least for some part with more extensive training, laboratory-based testing has to be available for backup. Second, in high-volume centers with a well-organized laboratory for genotyping, batch-wise testing using, in other words, TaqMan might be cost-efficient in comparison to POC genotyping. This should be analyzed in more detail. Third, when TaqMan genotyping is used, more SNP's and patients can be tested simultaneously, while the Spartan RX is limited to *CYP2C19**2, *3 and *17, for one patient at a time per genotyping device. Fourth, for low-risk patients, in other words, patients planned for elective PCI, it might not be necessary to have genotyping results available the same day and genotyping could actually be performed days or weeks in advance, in which batchwise laboratory-based testing can be a good alternative to POC testing.

CYP2C19 genotyping has additional costs. Current list prices for the SPARTAN RX system at time of publication are US\$200 (€162) per test kit, including testing for *2, *3 and *17, and US\$15,000 (€12,173) for the SPARTAN RX POC device. The costs for laboratory-based testing are estimated at €80 per patient, including reagent and personnel costs, but excluding equipment. The costs of laboratory based testing, however, may vary substantially per country and per laboratory site, in other words, with the number of tests performed. Nevertheless, a tailored antiplatelet strategy might be cost-effective, based on the much lower drug costs of generic clopidogrel (€14.40 per year in The Netherlands) compared with prasugrel and ticagrelor (€722 and €877 per year, respectively) [28]. This difference might be even bigger if a tailored approach would be able to reduce clinical events or give a gain in quality-adjusted life-years. In an observational study comparing 32 *CYP2C19* poor-metabolizer patients treated with clopidogrel compared with 41 poor-metabolizer patients treated with prasugrel in The Netherlands, a significant lower number of events was seen in the prasugrel-treated patient group [29]. Non-randomized trials showed that personalized antiplatelet treatment based on *CYP2C19* genotype might be able to reduce cardiovascular event rates and bleeding events [16,30]. Furthermore, several simulation studies showed superiority in cost-effectiveness for a *CYP2C19* genotype guided strategy compared with the use of prasugrel or ticagrelor, in both ACS and PCI patients [31–33]. In the ongoing Popular Genetics study, cost-effectiveness will be studied as a coprimary end point [23].

The putative gain-of-function allele *CYP2C19**17 has been associated with a lower platelet reactivity and an increased risk of bleeding [34,35]. In both the Popular Risk Score project and the Popular Genetics study, the *17 allele was not tested because it would not have had consequences for the treatment advice in those studies. Patients with *1/*1, *1/*17 or *17/*17 genotype would all be considered good responders and treated with clopidogrel, while patients with *2/*17 are classified as intermediate metabolizers and therefore treated with prasugrel or ticagrelor [34].

With genotyping becoming part of routine care, it is important to realize that the results might affect other treatments than just antiplatelet therapy, as it is involved in the metabolism of selective serotonin reuptake inhibitors, tricyclic antidepressants, proton pump inhibitors and voriconazole [22,36–38]. Therefore, it is important that the genotyping results are reported in the electronic patient file and that protocols that describe how to handle possible genotype–drug interactions, made in collaboration with the clinical pharmacist, are available. Genotyping results should also be shared with the local pharmacy and the general practitioner [22].

Limitations

The aim of this paper was to study the feasibility of different *CYP2C19* genotyping strategies in everyday practice, not to compare the time it takes to run the tests itself. When using the Spartan RX POC device, the time between collection of the buccal swab and test result will be around 1 h, while DNA extraction and TaqMan genotyping will take less than 2 h in total, when a blood sample is available.

Conclusion

It is feasible to perform *CYP2C19* genotyping within 24–48 h using on-site TaqMan genotyping or the Spartan RX POC device, making personalized medicine in antiplatelet therapy, based on pharmacogenetic data, feasible for everyday practice.

Summary points

- *CYP2C19**2 and *3 loss-of-function polymorphisms are clinically relevant predictors of clopidogrel efficacy in patients undergoing percutaneous coronary intervention.
- Alternative treatment options are available, using prasugrel or ticagrelor, for patients carrying a loss-of-function allele.
- In everyday practice, genotyping results can be obtained within 24–48 h in the majority of patients, using on-site TaqMan StepOnePlus or Spartan point-of-care devices.
- A personalized treatment strategy using *CYP2C19* genotyping in patients with planned percutaneous coronary intervention or with ST-segment elevation myocardial infarction is feasible.

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References

- 1 Ibanez B, James S, Agewall S *et al.* 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur. Heart J.* 39(2), 119–177 (2017).
- 2 Roffi M, Patrono C, Collet JP *et al.* 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur. Heart J.* 37(3), 267–315 (2016).
- 3 Wallentin L, Becker RC, Budaj A *et al.* Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* 361(11), 1045–1057 (2009).
- 4 Wiviott SD, Braunwald E, McCabe CH *et al.* Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N. Engl. J. Med.* 357(20), 2001–2015 (2007).
- 5 Siller-Matula JM, Lang I, Christ G, Jilma B. Calcium-channel blockers reduce the antiplatelet effect of clopidogrel. *J. Am. Coll. Cardiol.* 52(19), 1557–1563 (2008).
- 6 Gilard M, Arnaud B, Cornily JC *et al.* Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin. the randomized, double-blind OCLA (Omeprazole Clopidogrel Aspirin) study. *J. Am. Coll. Cardiol.* 51(3), 256–260 (2008).
- 7 Siller-Matula JM, Trenk D, Krahenbuhl S, Michelson AD, Delle-Karth G. Clinical implications of drug-drug interactions with P2Y12 receptor inhibitors. *J. Thromb. Haemost.* 12(1), 2–13 (2014).
- 8 Kazui M, Nishiya Y, Ishizuka T *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.* 38(1), 92–99 (2010).
- 9 Hulot JS, Bura A, Villard E *et al.* Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 108(7), 2244–2247 (2006).
- 10 Shuldiner AR, O'Connell JR, Bliden KP *et al.* Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA* 302(8), 849–857 (2009).
- 11 Mega JL, Close SL, Wiviott SD *et al.* Cytochrome P-450 polymorphisms and response to clopidogrel. *N. Engl. J. Med.* 360(4), 354–362 (2009).
- 12 Brandt JT, Payne CD, Wiviott SD *et al.* A comparison of prasugrel and clopidogrel loading doses on platelet function. magnitude of platelet inhibition is related to active metabolite formation. *Am. Heart J.* 153(1), 66.e9–66.e16 (2007).
- 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 Harmsze AM, van Werkum JW, Ten Berg JM *et al.* CYP2C19*2 and CYP2C9*3 alleles are associated with stent thrombosis. A case-control study. *Eur. Heart J.* 31(24), 3046–3053 (2010).
- 15 Sorich MJ, Rowland A, McKinnon RA, Wiese MD. CYP2C19 genotype has a greater effect on adverse cardiovascular outcomes following percutaneous coronary intervention and in Asian populations treated with clopidogrel: a meta-analysis. *Circ. Cardiovasc. Genet.* 7(6), 895–902 (2014).
- 16 Cavallari LH, Lee CR, Beitelshes AL *et al.* Multisite investigation of outcomes with implementation of CYP2C19 genotype-guided antiplatelet therapy after percutaneous coronary intervention. *JACC Cardiovasc. Interv.* 11(2), 181–191 (2017).
- 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 Brandt JT, Close SL, Iturria SJ *et al.* Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. *J. Thromb. Haemost.* 5(12), 2429–2436 (2007).
- 19 Scott SA, Sangkuhl K, Stein CM *et al.* Clinical Pharmacogenetics Implementation Consortium guidelines for CYP2C19 genotype and clopidogrel therapy. 2013 update. *Clin. Pharmacol. Ther.* 94(3), 317–323 (2013).
- 20 Holmes DR, Jr, Dehmer GJ, Kaul S, Leifer D, O'Gara PT, Stein CM. 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 endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons. *J. Am. Coll. Cardiol.* 56(4), 321–341 (2010).
- 21 European Medicines Agency (EMA). Summary of product characteristics (EPAR) – Clopidogrel (Plavix). 27th November 2017. www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000174/WC500042189.pdf
- 22 Swen JJ, Nijenhuis M, de Boer A *et al.* Pharmacogenetics. from bench to byte – an update of guidelines. *Clin. Pharmacol. Ther.* 89(5), 662–673 (2011).
- 23 Bergmeijer TO, Janssen PW, Schipper JC *et al.* CYP2C19 genotype-guided antiplatelet therapy in ST-segment elevation myocardial infarction patients-rationale and design of the patient outcome after primary PCI (Popular) Genetics study. *Am. Heart J.* 168(1), 16.e1–22.e1 (2014).
- 24 Sorich MJ, Vitry A, Ward MB, Horowitz JD, McKinnon RA. Prasugrel vs. clopidogrel for cytochrome P450 2C19-genotyped subgroups: integration of the TRITON-TIMI 38 trial data. *J. Thromb. Haemost.* 8(8), 1678–1684 (2010).

- 25 Janssen PWA. Selective intensification of P2Y₁₂ inhibitor therapy based on platelet reactivity, *CYP2C19* genotype and clinical risk factors in non-urgent PCI patients. In: *EuroPCR – Hot Line Session Antithrombotic Strategies*. European Society of Cardiology, Paris, France, 21–25 (2015).
- 26 Wirth F, Zahra G, Xuereb RG, Barbara C, Fenech A, Azzopardi LM. Comparison of a rapid point-of-care and two laboratory-based *CYP2C19**2 genotyping assays for personalisation of antiplatelet therapy. *Int. J. Clin. Pharm.* 38(2), 414–420 (2016).
- 27 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).
- 28 Zorginstituut Nederland. www.medicijnkosten.nl/
- 29 Deiman BA, Tonino PA, Kouhestani K *et al.* Reduced number of cardiovascular events and increased cost–effectiveness by genotype-guided antiplatelet therapy in patients undergoing percutaneous coronary interventions in The Netherlands. *Neth. Heart J.* 24(10), 589–599 (2016).
- 30 Sanchez-Ramos J, Davila-Fajardo CL, Toledo Frias P *et al.* Results of genotype-guided antiplatelet therapy in patients who undergone percutaneous coronary intervention with stent. *Int. J. Cardiol.* 225, 289–295 (2016).
- 31 Borse MS, Dong OM, Polasek MJ, Farley JF, Stouffer GA, Lee CR. *CYP2C19*-guided antiplatelet therapy: a cost–effectiveness analysis of 30-day and 1-year outcomes following percutaneous coronary intervention. *Pharmacogenomics* 18(12), 1155–1166 (2017).
- 32 Jiang M, You JH. *CYP2C19* LOF and GOF-guided antiplatelet therapy in patients with acute coronary syndrome: a cost–effectiveness analysis. *Cardiovasc. Drugs Ther.* 31(1), 39–49 (2017).
- 33 Kazi DS, Garber AM, Shah RU *et al.* Cost–effectiveness of genotype-guided and dual antiplatelet therapies in acute coronary syndrome. *Ann. Intern. Med.* 160(4), 221–232 (2014).
- 34 Harmsze AM, van Werkum JW, Hackeng CM *et al.* The influence of *CYP2C19**2 and *17 on on-treatment platelet reactivity and bleeding events in patients undergoing elective coronary stenting. *Pharmacogenet. Genomics* 22(3), 169–175 (2012).
- 35 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).
- 36 Hicks JK, Swen JJ, Thorn CF *et al.* Clinical Pharmacogenetics Implementation Consortium guideline for *CYP2D6* and *CYP2C19* genotypes and dosing of tricyclic antidepressants. *Clin. Pharmacol. Ther.* 93(5), 402–408 (2013).
- 37 Hicks JK, Bishop JR, Sangkuhl K *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for *CYP2D6* and *CYP2C19* genotypes and dosing of selective serotonin reuptake inhibitors. *Clin. Pharmacol. Ther.* 98(2), 127–134 (2015).
- 38 Moriyama B, Obeng AO, Barbarino J *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for *CYP2C19* and voriconazole therapy. *Clin. Pharmacol. Ther.* doi:10.1002/cpt.583 (2016) (Epub ahead of print).