Genotyping of CYP2C19 helps to reassure sufficient effects of antiplatelet therapy in combination with proton pump inhibitors (PPIs)

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Abstract

Clopidogrel therapy in combination with proton pump inhibitors (PPIs) is controversial in the treatment for ischemic heart diseases after coronary stent implantation because PPIs (especially omeprazole) are suggested to attenuate the antiplatelet effect of clopidogrel. The polymorphic CYP2C19 enzyme has a pivotal function in the metabolism of both agents. In addition, omeprazole can irreversibly inhibit the activity of CYP2C19 enzyme. The frequency of CYP2C19 polymorphisms with a poor metabolizer phenotype is especially high (29-35%) in the Asian population. The present study investigated whether CYP2C19 genotyping is required in clopidogrel-treated patients with concomitant omeprazole treatment for genetically at risk poor metabolizing CYP2C19 populations. In this study literature was searched and reviewed which comprised the specific research area. The results indicated that omeprazole in combination with clopidogrel is related to a higher recurrence risk of adverse cardiovascular events and a higher mortality rate. Interestingly, the antiplatelet effect of clopidogrel was more affected by omeprazole in homozygous CYP2C19*1 carries than in heterozygous (*1/2 or *1/3) and homozygous (*2/2 or *2/3) CYP2C19 carriers. Therefore, CYP2C19 genotyping is advised in clopidogrel-treated patients with concomitant omeprazole treatment. This to prevent adverse cardiovascular events in clopidogrel treated patients in combination with PPIs. However, the number of studies is rather limited up till now in relation to the CYP2C19 status and the combination of omeprazole plus clopidogrel. Therefore further clinical studies are indispensable to verify the conclusion in this study.

Contents

Introduction

The dual antiplatelet thienopyridines (e.g., prasugrel, ticlopidine and clopidogrel) and aspirin (acetylsalicylic acid) therapy is widely prescribed to prevent the adverse recurrence of coronary heart diseases, acute coronary diseases and angina pectoris (from now on defined as cardiovascular events). The dual antiplatelet therapy is recommended for up to 12 months after coronary stent implantation due to a higher risk of stent thrombosis with adverse outcome in the first one year. [1] The relative risk recurrence of cardiovascular events was higher in patients treated with aspirin alone when compared with clopidogrel treatment. These findings suggest that clopidogrel is more effective when compared with aspirin alone. The combination of clopidogrel with aspirin is more effective in antiplalete aggregation treatment than either agent alone. [2] Adverse clinical side effects in dual antiplatelet therapy are abdominal discomfort or pain, nausea, dyspepsia, and gastrointestinal hemorrhage. Aspirin irreversibly inhibits gastric mucosal cyclooxygenase 1 (COX-1) activity which consequently suppresses mucosal protective effects of prostaglandins. [3] The gastrointestinal side-effects of clopidogrel are presumably due to the antiplatelet activity which causes existing lesions to bleed. In 2008, the use of prophylactic proton pump inhibitors (PPIs) in combination with dual antiplatelet therapy was specified in a clinical consensus guideline by the American College of Cardiology Foundation (ACCF) amongst others.[4] However, some studies suggested that the antiplatelet effect of clopidogrel is attenuated by the administering of PPIs. [5-7] In 2009, the Food and Drug administration (FDA) and the European Medicines Agency (EMA) issued a warning to avoid concomitant intake of clopidogrel with PPIs, especially omeprazole. [8,9] In reaction to the warning from the FDA and EMA, the clinical expert consensus from 2008 was adjusted in March 2010. [10] Clopidogrel is majorly activated by CYP2C19 a member of the cytochromes P450 (CYPs) enzymes which play a pivotal role in drug metabolism either in bioactivation or deactivation. The studies hitherto did not include the effect of interindividual CYP2C19 genetic variability. [7] The frequency of CYP2C19 polymorphisms with a poor metabolizer phenotype is especially high (29-35%) in the Asian population when compared to the Caucasian or African population (15%). [11] The hypothesis in this study was, that the clopidogrel and omeprazole interaction is negatively affected by CYP2C19 polymorphisms. Thus, it would be more likely that poor metabolizers have a higher recurrence risk of cardiovascular events when compared with the extensive metabolizer phenotype. Therefore, the purpose of this study was to investigate whether CYP2C19 genotyping is useful in clopidogrel-treated patients with concomitant omeprazole treatment. To this end, PubMed was searched for the terms omeprazole plus clopidogrel and combined with a separate search of these individual terms with the terms metabolism, pharmacokinetics, CYP2C19, and polymorphisms.

Pharmacokinetics- and dynamics of omeprazole

Omeprazole and other PPIs are weak bases which consist of a heterocyclic pyridine and a benzimidazole moiety linked via a methylsulfinyl group. The plasma half-life time (~ 1-2 h) of omeprazole does not correlate to its response (~ 28 h) due to the irreversible inhibition of the proton pump. [12] The prodrug form of omeprazole is absorbed in the small intestines into the blood circulation, where after it passes the cell membrane of the parietal cell. The pyridine and benzimidazole moiety undergo a protonation step in the acid (pH ~ 1.0) environment of the luminally located canaliculi in the parietal cells. This chemical rearrangement culminates in an active tetracyclic cation, which can covalently reacts with cysteine 813 by a disulfide formation on the hydrogen-potassium adenosine triphosphate (H^+/K^+-ATPase) pump. This binding prevents gastric acid secretion into the lumen resulting in an increased intragastric pH. The activation of omeprazole is influenced by the ionization constant (p*Ka*) and the environmental pH. Omeprazole is largely metabolized by the isoenzyme CYP2C19 (*S*-mephenytoin-hydroxylase) and CYP3A4 (nifedipine hydroxylase) of the cytochrome P450 (CYP) system. Omeprazole is metabolized (see figure 1, page 11) by CYP2C19 and CYP3A4 respectively to 5-hydroxyomeprazole and 5-hydroxyomeprazole-sulfone. Another less frequently utilized metabolic pathway is the conversion of omeprazole to sulfone by CYP3A4. The sulfone is further converted to 5-hydroxyomeprazole-sulfone by CYP2C19. [6] Omeprazole and other common PPIs (e.g., lansoprazole, pantoprazole and esomeprazole) competitively inhibit the CYP2C19 enzyme activity. This results in an attenuated catalytic enzyme activity. Omeprazole has a higher (K_i 2-6 mM) inhibitory potency on CYP2C19 when compared with its S-isomer (K_i 8 mM) on CYP2C19 activity. [13] About 77% of the oral omeprazole dose is excreted urinary. [14]

Pharmacokinetics- and dynamics of clopidogrel

The prodrug clopidogrel bisulfate (Plavix®, Grepid® or Iscover ®) is rapidly absorbed in the intestines. The oxidation to its active metabolite (see Figure 1, page 11) is achieved by CYP1A2, CYP2B6, CYP2C9, CYP2C19 and, CYP3A4/5. Clopidogrel is first converted to the intermediate 2-oxo-clopidogrel followed by the formation of the active metabolite 2-oxoclopidogrel. The relative contribution of the CYP2C19 enzyme in the first and second phase of the activation is respectively 44.9% and 20.6%. In the second metabolic phase, the CYP3A4 enzyme plays a the major (39.8%) role in the metabolic activation. [15] The involvement of the CYP2C19 enzyme in drug metabolism varies from 58% to 67% in intermediate metabolizers and from 56% to 74% in ultrarapid metabolizers. [16,17] The majority (~ 85%) of the prodrug is hydrolyzed to its inactive form by hepatic carboxylesterases. [18] Platelet activation occurs upon endothelial vessel injuries which release coagulation proteins such as Von Willebrand factor (vWF), collagen, and vitronectin among others.

The platelet dense granules release ADP molecules and interact with the G_q-coupled receptor P2Y1. This interaction induces morphological changes and the phospholipase C activity which increases the cytosolic calcium ion levels. The G_i-coupled P2Y12 ADP receptor, inhibits the enzymatic activity of adenylyl cyclase. [19] The agonist interaction with the P2Y12 receptor contributes mainly to the stabilization of platelet aggregation induced by thrombin or thromboxane A_2. [20] The remaining small percentage (~ 15%) of the active metabolite 2-oxoclopidogrel irreversibly inhibits the G-protein coupled receptor P2Y12 by binding its cysteine residues. [21] The inhibition of ADP-mediated platelet aggregation culminates in an antithrombotic effect.

Clopidogrel and omeprazole interaction

The omeprazole clopidogrel aspirin (OCLA) study was the first prospective trial to evaluate the effect of omeprazole on the efficacy of clopidogrel. The study was based on observational results from 2006. [22] The OCLA study included patients with coronary stent implants which were randomized to receive either omeprazole or placebo. The results indicated an attenuated clopidogrel response in the omeprazole treated patients when compared with the placebo-treated patients. [23] In another prospective randomized study by Cuisset, et al. from 2009, patients received either omeprazole or pantoprazole in combination with their dual antiplatelet therapy. The omeprazole group indicated more clopidogrel nonresponders than the pantoprazole group. [24] In another randomized crossover trial were similar results obtained in comparison with the previous study from 2009. [25] In consistence with the previous studies Angiollilo, et al. investigated whether the interaction between clopidogrel and omeprazole could be mitigated by separate administer with 12 hours apart. The pharmacokinetic results indicated a decrease in the clopidogrel plasma concentration. The maximal platelet aggregation induced by ADP was decreased when compared with the simultaneous administer of the agents. In addition, the increase of the daily loading dose of clopidogrel did not affect the clopidogrel plasma concentration and maximal platelet aggregation. [26] However, the drug interaction between omeprazole and clopidogrel is not fully consistent. The study by Gremmel, et al. found no differences in the antiplatelet effect of clopidogrel either with or without omeprazole. [27] The *ex vivo* mechanistic evidence of an omeprazole and clopidogrel interaction was in consistence with several clinical retrospective population-based cohort studies. In the study by Ho, et al. included 8,205 patients with clopidogrel treatment. The majority (~ 64%) of the group had concomitantly PPI treatment. The study indicated a higher (~ 21%) mortality and rehospitalization rate for acute coronary syndromes when compared with the patients without PPI treatment. [28] Another large scale retrospective study was conducted by Kreutz, et al. including almost 17,000 patients treated with clopidogrel either with (~ 41%) or without (~ 59%) omeprazole concomitant. After one year the patients treated with clopidogrel in combination with a PPI indicated a higher rate of major cardiovascular events with an adjusted hazard ratio of > 1,5 than patients treated with clopidogrel alone.

The associations were similar for each PPI including omeprazole, esomeprazole, and pantoprazole. [29] The cardiovascular recurrence risk within 90 days after hospital discharge was higher (40%) in patients treated with clopidogrel and PPI when compared with clopidogrel alone. [30] Other retrospective studies included 800 up to 10,000 patients treated with either clopidogrel alone or combined with PPIs indicated a higher (13,8% to 41%) incidence of major adverse cardiovascular events. The cardiovascular mortality rate was higher (4,7%) in the combination therapy. Although one study found slight (p = 0,51) similar mortality rates between patients who received clopidogrel alone or combined with PPIs. [31-33] The higher incidence of adverse cardiovascular events and cardiovascular-related deaths in clopidogrel in combination with PPIs was confirmed in a meta-analysis including about 160,000 patients. Interestingly, pantoprazole alone was associated with an increased risk but not omeprazole or other PPIs. [34] The previously summarized findings of all these studies are consistent with each other.

However, other retrospective and prospective population-based cohort studies indicated inconsistency with the previously mentioned studies. The posthoc study by Dunn, et al. indicated no association between PPIs and adverse cardiovascular events. [35] This was confirmed by a retrospective study including about 20,000 clopidogrel-treated patients in combination with either pantoprazole (62%) or omeprazole (9%). [36] In 2009, a study was conducted including about 19,000 patients treated with clopidogrel either with or without PPI indicated no increased risk between the both groups. Although elderly (≥ 65 years) patients had a slightly increased rate of cardiovascular event or mortality it was claimed not to have any clinical importance. [37] In 2013, Kwok, et al. performed a meta-analysis of a total of 22 studies including over 200,000 individuals. The study indicated no differential cardiovascular risk between clopidogrel either without or in combination with PPIs. [38]

CYP2C19 loss-of-function variants in clopidogrel and omeprazole metabolism

The hepatic CYP monooxygenase enzyme system has a pivotal function in the metabolism of clinically important drugs. The polymorphic enzyme CYP2C19 (Cytochrome P450, Family 2, Subfamily C, Polypeptide 19) is a member of the CYP enzyme system genetically located within a cluster on chromosome 10q24. The CYP2C19 gene has more than 30 allelic variants which are demographically spread over ethnic groups worldwide (http://www.cypalleles.ki.se/cyp2c19.htm). The CYP2C19*2 (c.681G>A; rs4244285) null allele is the most frequent (15% in Caucasians and Africans; 29-35% in Asians) among poor metabolizers (PM). The splicing defect in exon 5 due to the point mutation terminates the protein synthesis too early. The CYP2C19*3 (c.636G>A; rs4986893) is more common among Asians (2-9%) than other ethnical groups. A single base transition results in a premature stop codon which culminates in a truncated protein.

The frequency of one or two CYP2C19 loss of function alleles accounts for respectively 42.5% and 10% in the Asian population. In comparison, in the Caucasian population this percentage is much lower, respectively 25.5% and 2.4%. [39] The PM genotype affects the metabolism of some antidepressants and anticonvulsant. [6,11]

In 2007, a study was performed to determine the pharmacokinetic and pharmacodynamics responses to clopidogrel in homozygous metabolizers extensive (homEM; *1/1) and heterozygous extensive (hetEM; *1/2) and poor metabolizers (PM *2/2). The CYP2C19*2 loss of function allele indicated a less total drug exposure over time and inhibition of the platelet aggregation (IPA) (see table 1) compared to the homEM. The maximum serum concentration (C_{max}) active metabolite clopidogrel was lower in the hetEM and PM group when compared to the WT. The antiplatelet agent prasugrel did not indicate the same results as clopidogrel in the PM genotype. [40] A more recently performed clinical study under 642 Chinese individuals with clopidogrel treatment to study the association between CYP2C19*2/*3 and the occurrence of cardiovascular events over one year. The CYP2C19*2 and *3 mutant allele carriers had a higher ADP-induced maximal platelet activation than homozygous WTs. There was a higher incidence of stent thrombosis in mutant allelic variants than in the noncarriers. [41] Thus, CYP2C19*2 or *3 allelic variant affects the efficacy of clopidogrel. The recurrence risk of cardiovascular events is increased in the CYP2C19*2 and *3 allelic variance under patients who receive clopidogrel. This was confirmed by a recent meta-analysis study under Asians and Westerns from 2015. [39] In addition, the genetic effect on the function of clopidogrel is rather an agent than class specific.

The hepatic metabolism of omeprazole is mainly (>80%) performed by the CYP2C19 enzyme. Shirai, et al. investigated whether CYP2C19 genotypic status affected the metabolism of omeprazole and rabeprazole by measuring the intragastric pH. The homEM (*1/1), hetEM (*1/2 and *1/3) and PM (*2/3 and *2/2) indicated differences (see table 1) in the intragastric pH between each group. The pH values were the highest in the CYP2C19*3 allele carrying group followed by the CYP2C19*2 allele in single and repeated omeprazole administer. In the PM, the area under the curve plasma concentration (AUC) over 24 hours was higher for omeprazole and its metabolite sulfone which is converted by CYP3A4 when compared with the hetEM and homEM. The concentration of the 5-hydroxyomeprazole metabolite converted by CYP2C19 was lower. Rabeprazole showed no difference in intragastric pH values between the genetic groups. [42] Thus, CYP2C19 deficiency causes sustained omeprazole plasma concentrations resulting in an extended inhibition of gastric acid secretion when compared to the other metabolic phenotypes.

Table 1 The clopidogrel and omeprazole pharmacokinetic and pharmacodynamic characteristics in different CYP2C19 genotypes.

	Clopidogrel [I]			Omeprazole [II]	
CYP2C19	**AUC_{0-24h} (ng h mL^{-1})**	**C_{max} (ng h mL^{-1})**	**IPA (at 4h)**	**AUC_{0-24h} (ng h mL^{-1})**	**Intragastric pH**
***1/1**	76.2 ± 17.9	58.4 ± 9.2	39.1 ± 3.4%	523.5 ± 120.4	2.3
***1/2 *1/3**	41.5 ± 5.7	35.3 ± 4.3	20.3 ± 3.9%	1095.6 ± 144.2	3.3
***2/2 *2/3**	26.9	27.9	3.8%	5606.8 ± 1055.7	4.1

The data was extracted from Brandt, et al., 2007 [40] and Shirai, et al., 2001[42]

[I] Repeated dose (300 mg) clopidogrel [II] single dose (20 mg) omeprazole.

Potential interaction mechanism of omeprazole and clopidogrel

Several clinical studies suggested that omeprazole attenuates the efficacy of clopidogrel. [39] The underlying clinical drug interaction mechanism (see Figure 1) between omeprazole and clopidogrel is still not completely understood. In a study by Ohbuchi et al., the bioactivation of 2-oxo-clopidogrel to its active metabolite was studied *in vitro* with recombinant human CYP isoenzymes. To stabilize the thiol active form of clopidogrel it was derivatized and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results showed that omeprazole inhibited the bioactivation of 2-oxo-clopidogrel to its active metabolite for CYP2B6, CYP3A4, and CYP2C19 in a concentration-dependent manner. The half maximal inhibitory concentration (IC_{50}) of omeprazole for CYP2C19 was less when compared with the other isoenzymes. The metabolic activity of CYP2C19 enzyme was also inhibited by other PPIs (lansoprazole, esomeprazole, pantoprazole, and rabeprazole). The gastric acid suppressing H_2-receptor antagonist famotidine inhibited the clopidogrel metabolic activation slightly. [43] Another study by Boulenc et al. performed both a static and dynamic model analysis on physiologically based pharmacokinetics, to study *in vitro* the relative inhibition of CYP2C19 by omeprazole. This was performed in human liver microsomes (CYP2C19 intermediate (IM), extensive (EM) and ultrarapid (UM) metabolizers) preparations. A time-dependent inhibition assay indicated a hyperbolic relationship between the inhibition constant (K_i), the rate of CYP2C19 inactivation (K_{inact}) and the omeprazole concentration. CYP2C19 involvements were estimated to be from 58 to 67% in IM, from 58 to 72% in EM, and from 56 to 74% in UM. The inhibitory parameters of omeprazole in combination with the CYP2C19 relative estimated contribution to the formation of the active metabolite was predicted at 80-mg omeprazole dose in EM, IM, and UM. [17] Ogilvie et al. showed similar results in their time-dependent inhibition assay. In an assessment of inhibition reversibility by ultracentrifugation, the residual activity of CYP2C19 was not reserved. The result indicated an irreversible or quasi-irreversible inhibition of the CYP2C19 enzyme by omeprazole. [44] In general, the studies concluded that the reduced efficacy of clopidogrel is due to the irreversible mechanism-based inhibitor function of omeprazole for the CYP2C19 enzyme.

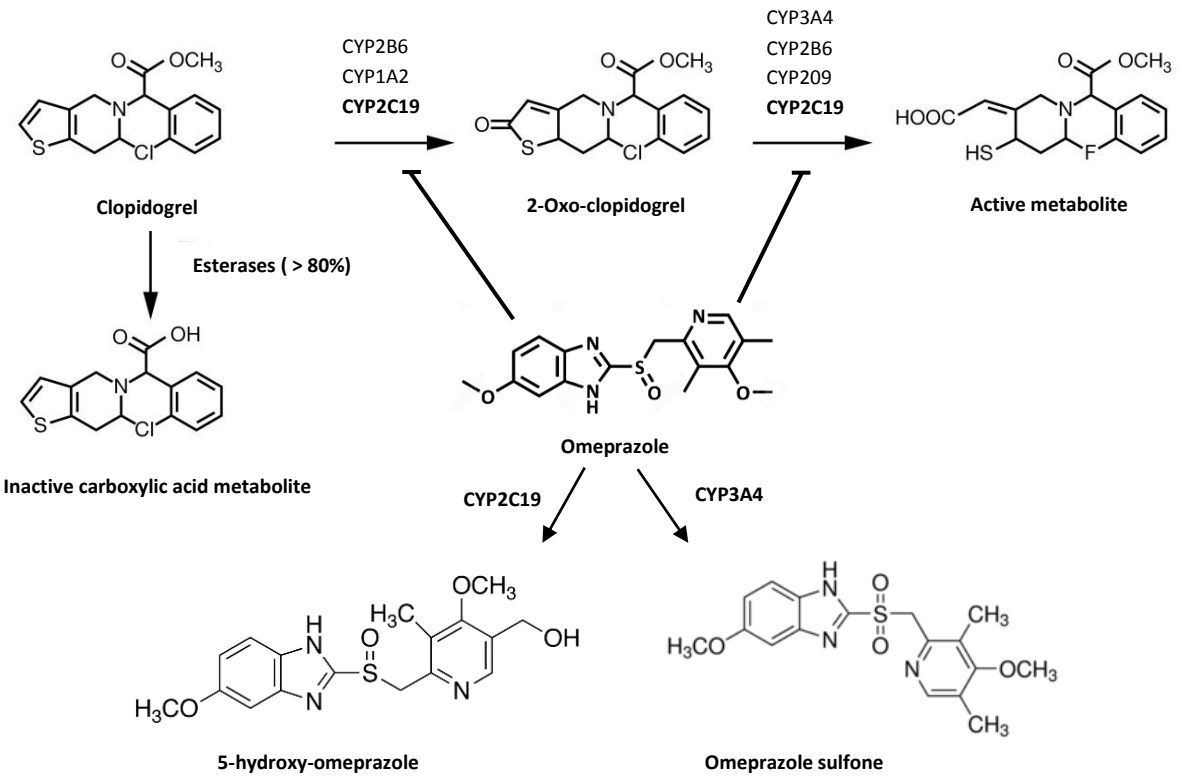

Figure 1 The metabolism of clopidogrel and omeprazole by CYP isoenzymes including a potential interaction mechanism for omeprazole by inhibition of CYP2C19 enzyme.

The effect of omeprazole on clopidogrel activity in CYP2C19 poor metabolizers

As stated previously, the efficacy of clopidogrel might be attenuated by omeprazole with a higher risk of adverse cardiovascular events or even mortality. However, the potential influence of CYP2C19 genetic interindividual variability was not included in these particular studies. The study by Furuta et al. was the first that investigated the effect of PPIs on the antiplatelet function in relation to the variability in CYP2C19 genotypes. In this single-arm crossover, study, individuals received clopidogrel for seven days before starting the PPI treatment including omeprazole, lansoprazole, and rabeprazole. The inhibition of platelet aggregation by clopidogrel was not changed by any PPI in the whole group. The aggregation inhibition in CYP2C19 heterozygous extensive metabolizers (*1/2, *1/3, or *2/3 genotype) was not decreased (see table 2) with omeprazole therapy when compared with the clopidogrel treatment alone. Interestingly, homozygous extensive metabolizers (*1/1 genotype) showed in the study a decreased inhibition (see table 2) of the ADP-induced platelet aggregation in the concomitant therapy. The decrease in the homEM group was not classified as the clopidogrel low-responder (< 30% IPA). The inhibition of platelet aggregation in homEMs was attenuated by omeprazole and clopidogrel administration in the morning but appeared to be improved when omeprazole was dosed 12 hours later. This was not the case in the hetEM group. [45] Another study by Liu et al. including 142 healthy individuals indicated similar results (see table 2) for poor metabolizers (PM; *2/3, or *2/2), heterozygous extensive metabolizers (hetEMs; *1/2, or *1/3) and homozygous extensive metabolizers (homEMs; *1/1) in dual antiplatelet therapy in combination with omeprazole. The prevalence of high residual platelet reactivity on clopidogrel was higher (68%) in the PM when compared with the other groups. In randomized individuals without omeprazole was a better response to clopidogrel. The ADP-induced aggregation between individuals with or without omeprazole was the largest in homEMs when compared to the other genetic groups. In addition, the frequency of nonresponders to clopidogrel in combination with omeprazole was higher when compared to the group without omeprazole. [46] The study by Hokimoto et al. from 2014 examined the influence of rabeprazole on the antiplatelet function of clopidogrel in relation to several CYP2C19 genotypes. Rabeprazole is metabolically converted in a non-enzymatic manner with a lower potent inhibition of CYP2C19 when compared with omeprazole. In clopidogrel-treated patients, the platelet reactivity and adverse cardiovascular event rate were lower in the EM group when compared to the IM and PM group. Individuals with clopidogrel treatment either with or without rabeprazole did not indicate differences in platelet reactivity and adverse clinical events in three genotypic groups. [47] Other PPIs such as lansoprazole, pantoprazole, and esomeprazole in combination with clopidogrel did not affect the platelet inhibition in different CYP2C19 genotypes. [48,49] The most recent observational study regarding clopidogrel and omeprazole in CYP2C19 polymorphisms was dated from 2011. The current status of the study is unknown. [50]

Table 2 **The results of the ADP-induced platelet aggregation (IPA) in CYP2C19 genotyped individuals with clopidogrel treatment either with or without omeprazole from two independent studies.**

	IPA (%)				
CYP2C19	**Clopidogrel**		**Δ_{IPA}**	**p-value**	**Reference**
	No PPI	**Omeprazole**			
***1/1**	58.3	51.2	7.7	0.015	Furuta et al., 2010 [45]
***1/2** ***1/3** ***2/3**	36.6	33.3	3.3	0.443	
***1/1**	45.7 ± 14.2	35.5 ± 16.0	10.2	0.028	Liu et al., 2012 [46]
***1/2** ***1/3**	47.2 ± 13.1	41.4 ± 14.4	5.8	0.085	
***2/2** ***2/3**	53.9 ± 13.0	52.9 ± 15.0	1.0	0.864	

Discussion

The findings suggest that CYP2C19 genotyping useful in clopidogrel-treated patients with concomitant omeprazole treatment in order to identify poor metabolizing population as well as in extensive metabolizing populations. This to reassure a sufficient effect of antiplatelet therapy in combination with PPIs, and thus prevent the occurrence of adverse cardiovascular events. A significant number of retrospective and prospective population-based cohort studies investigated the potential association between omeprazole in relation to the attenuated functional effect of the antiplatelet agent clopidogrel. The combined study results confirm a relation between clopidogrel treatment in combination with omeprazole and an increased recurrence risk of major cardiovascular events. These results were confirm previous *ex vivo* and *in vitro* pharmacological observations. The clopidogrel efficacy was more affected in CYP2C19*2 and CYP2C19*3 loss of function allele carriers when compared with the wildtype allele group. Conversely, omeprazole was more efficient in the loss of function alleles than in the wildtype group. However, the studies hitherto did not include the effect of interindividual CYP2C19 genetic variability in relation to clopidogrel treatment in combination with omeprazole. Interestingly, the platelet aggregation was reduced in homozygous CYP2C19*1 carriers who received clopidogrel treatment concomitant with omeprazole when compared with the heterozygous (CYP2C19*1, *2 or *3) carriers. This results partially rejects the previously defined hypothesis that the clopidogrel and omeprazole interaction is negatively affected by CYP2C19 polymorphisms. Therefore it would be more likely that poor metabolizers have a higher recurrence risk of cardiovascular events when compared with the extensive metabolizer phenotype.

The following explanations can be offered for the reason why clopidogrel was more affected in reduced function CYP2C19 alleles. The metabolic activation of clopidogrel is dependent upon CYP isoenzymes especially CYP2C19 and CYP3A4. The reduced function of the enzymes might affect the activation of clopidogrel with negative consequence for the efficacy of the antiplatelet function. The omeprazole efficacy measured by the intragastric pH was lower in the homozygous CYP2C19*1 metabolizers when compared with the other genotypes. The high rate of metabolic activity could be an explanation for these observations. The Dutch Pharmacogenetics Working Group recommens a higher omeprazole dose for the ultrarapid metabolizer CYP2C19*17/17 genotype in the eradication of the gut bacteria *Helicobacter Pylori* based on the highly increased metabolism. [51] The following interpretation could be offered as reason why homozygous CYP2C19*1 carriers were relative more affected by clopidogrel treatment in combination with omeprazole when compared with heterozygous carriers. The increased metabolic rate of the CYP2C19*1/1 enzyme variant might be more inhibited due to a higher potential affinity of omeprazole for this particular enzyme when compared with other polymorphic CYP2C19 enzymes variants.

The higher affinity of omeprazole for the functional more active enzyme consequently inhibits the metabolic activation of clopidogrel more. Therefore, the relative maximal platelet aggregation is more affected in the CYP2C19*1/1 when compared with other CYP2C19 genetic variants. Conversely, the reduced functional CYP2C19 enzyme variants might be less affected by the inhibitory effect of omeprazole. This may result in a less affected clopidogrel activation which presumably has fewer implications for the relative antiplatelet aggregation functionality of clopidogrel.

The CAPRIE (clopidogrel versus aspirin in patients at risk of ischaemic events) randomized blinded trial including about 20.000 patients found slightly less (1.99%) gastrointestinal adverse events for clopidogrel monotherapy when compared with aspirin (2.66%). [52] Therefore, it may be assumed that PPI prescription is only necessary after clinical complications of gastrointestinal events. The use of other gastric acid inhibitor like the histamine H_2-receptor antagonist famotidine was reported to prevent gastrointestinal damage. This gastric acid inhibitor has little or no effect on CYP isoenzymes and consequently does not attenuated the efficacy of clopidogrel. [53]

The importance of these findings might have clinical implications for the clopidogrel-treatment in combination with omeprazole. In a pan-ethnic analysis including Caucasian, African American, Hispanic, and Ashkenazi Jewish populations more than 40% was classified as homozygous CYP2C19*1 carrier. The poor metabolizer phenotype was lower in this pan-ethnic analysis. [54] In Japanese, Chinese and Koreans the poor metabolizer phenotype is much higher than that reported in the Caucasian population. It is imported to remember that an estimated 23.6 million persons per year will be affected by cardiovascular diseases worldwide by the year 2030 with an annual cost of 316.6 billion dollars. [55] A cost-effectiveness study showed that genotype-guided antiplatelet therapy may be more cost-effective when compared to the selection without regard to the CYP genotype status of the patient. [56] New studies are indispensable to optimize the cardiovascular therapies and reassure the effectivity of the current therapies.

The results in this study most be interpreted within the limitations of the available studies. The clinical effects of omeprazole on the activity of clopidogrel in interindividual CYP2C19 variability is studied rather limited. The studies by Furuta et al. and Liu et al. investigated presumably only Asian population in a rather limited amount. The statistical calculations were not adjusted to a wider ethnical population. In the studies only *ex vivo* samples were analyzed on platelet aggregation. Up to now there are no retrospective or prospective population-based cohort studies who investigated the consistency of the *ex vivo* results. Consequently it is not possible to relate clopidogrel treatment in combination with omeprazole in homozygous CYP2C19*1 carriers to a different adverse cardiovascular rate when compared with other genotypes. The study by Furuta et al. did not include the patients treated with aspirin which is specified by clinical consensus in clopidogrel treatment. A possible additional effect of aspirin was not excluded in relation to CYP2C19 status and omeprazole plus clopidogrel.

An *in vivo* study concluded that low-dose aspirin induced the activity of the CYP2C19 enzyme. Aspirin is mainly metabolized by nonspecific esterases in the liver but not by the CYP isoenzymes. [57] In addition, several large scale population-based studies indicated no association between clopidogrel treatment with omeprazole in relation to adverse cardiovascular events. [38] These results should not be neglected.

Further additional research studies must be carried out to investigate the association between CYP2C19 genotype status in relation to clopidogrel metabolism in combination with PPIs. It might be especially interesting to study the less common ultrarapid metabolizer CYP2C19*17 allele in relation to clopidogrel treatment with omeprazole. In addition, large scale prospective population-based studies might contribute to reveal clinical implications and usefulness of CYP2C19 genotyping in antiplatelet therapy.

In conclusion, clinical practitioners should be aware of the possible adverse consequences of the combined treatment with PPIs, especially omeprazole. The necessity of PPIs in antiplatelet therapy should be reconsidered. However, the efficacy of antiplatelet therapy with or without PPIs is mainly based on CYP2C19 genotype status. Therefore, genotype-guided antiplatelet therapy should be discussed to avoid unnecessary adverse cardiovascular risks.

References

1. Pang S, Shi SY, Zhang YJ, et al. The impact of dual antiplatelet therapy duration on primary composite endpoint after drug-eluting stent implantation: A meta-analysis of 10 randomized trials. *Int J Cardiol*. 2016;202:504-506.

2. Albers GW, Amarenco P. Combination therapy with clopidogrel and aspirin: Can the CURE results be extrapolated to cerebrovascula patients? *Stroke*. 2001;32(12):2948-2949.

3. Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. *J Clin Biochem Nutr*. 2011;48(2):107-111.

4. Bhatt DL, Scheiman J, Abraham NS, et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: A report of the american college of cardiology foundation task force on clinical expert consensus documents. *Circulation*. 2008;118(18):1894-1909.

5. Laine L, Hennekens C. Proton pump inhibitor and clopidogrel interaction: Fact or fiction? *Am J Gastroenterol*. 2010;105(1):34-41.

6. Hagymasi K, Mullner K, Herszenyi L, Tulassay Z. Update on the pharmacogenomics of proton pump inhibitors. *Pharmacogenomics*. 2011;12(6):873-888.

7. Scott SA, Owusu Obeng A, Hulot JS. Antiplatelet drug interactions with proton pump inhibitors. *Expert Opin Drug Metab Toxicol*. 2014;10(2):175-189.

8. FDA Center for Drug Evaluation and Research (CDER). Interaction between esomeprazole/omeprazole and clopidogrel label change. http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm327922.htm. Updated 2012. Accessed 01/08, 2016.

9. European Medinices Agency. Public statement on possible interaction between clopidogrel and proton pump inhibitors. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2009/11/news_detail_000194.jsp&mid=WC0b01ac058004d5c1. Updated 2012. Accessed 01/08, 2016.

10. Abraham NS, Hlatky MA, Antman EM, et al. ACCF/ACG/AHA 2010 expert consensus document on the concomitant use of proton pump inhibitors and thienopyridines: A focused update of the ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: A report of the american college of cardiology foundation task force on expert consensus documents. *Circulation*. 2010;122(24):2619-2633.

11. Scott S, Sangkuhl K, Gardner E, et al. Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. *Clin Pharmacol Ther*. 2011;90(2):328-332.

12. Sachs G, Shin JM, Howden CW. Review article: The clinical pharmacology of proton pump inhibitors. *Aliment Pharmacol Ther*. 2006;23 Suppl 2:2-8.

13. Li XQ, Andersson TB, Ahlstrom M, Weidolf L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole on human cytochrome P450 activities. *Drug Metab Dispos*. 2004;32(8):821-827.

14. Vanderhoff BT, Tahboub RM. Proton pump inhibitors: An update. *Am Fam Physician*. 2002;66(2):273-280.

15. 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*. 2010;38(1):92-99.

16. Simon T, Bhatt DL, Bergougnan L, et al. Genetic polymorphisms and the impact of a higher clopidogrel dose regimen on active metabolite exposure and antiplatelet response in healthy subjects. *Clin Pharmacol Ther*. 2011;90(2):287-295.

17. Boulenc X, Djebli N, Shi J, et al. Effects of omeprazole and genetic polymorphism of CYP2C19 on the clopidogrel active metabolite. *Drug Metab Dispos*. 2012;40(1):187-197.

18. Sangkuhl K, Klein TE, Altman RB. Clopidogrel pathway. *Pharmacogenet Genomics*. 2010;20(7):463-465.

19. Woulfe Donna. ADP and platelets: The end of the beginning. *J Clin Invest*. 2001;107(12):1503.

20. Cattaneo M., cattaneo. The platelet P2Y12 receptor for adenosine diphosphate: Congenital and drug-induced defects. *Blood*. 2011;117(7):2102.

21. Farid NA, Kurihara A, Wrighton SA. Metabolism and disposition of the thienopyridine antiplatelet drugs ticlopidine, clopidogrel, and prasugrel in humans. *J Clin Pharmacol*. 2010;50(2):126-142.

22. Gilard M, Arnaud B, Le Gal G, Abgrall JF, Boschat J. Influence of omeprazol on the antiplatelet action of clopidogrel associated to aspirin. *Journal of thrombosis and haemostasis : JTH*. 2006;4(11):2508-9.

23. 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*. 2008;51(3):256-60.

24. Cuisset T, Frere C, Quilici J, et al. Comparison of omeprazole and pantoprazole influence on a high 150-mg clopidogrel maintenance dose the PACA (proton pump inhibitors and clopidogrel association) prospective randomized study. *J Am Coll Cardiol*. 2009;54(13):1149-53.

25. Fontes-Carvalho R, Albuquerque A, Araujo C, Pimentel-Nunes P, Ribeiro VG. Omeprazole, but not pantoprazole, reduces the antiplatelet effect of clopidogrel: A randomized clinical crossover trial in patients after myocardial infarction evaluating the clopidogrel-PPIs drug interaction. *Eur J Gastroenterol Hepatol*. 2011;23(5):396-404.

26. Angiolillo DJ, Gibson CM, Cheng S, et al. Differential effects of omeprazole and pantoprazole on the pharmacodynamics and pharmacokinetics of clopidogrel in healthy subjects: Randomized, placebo-controlled, crossover comparison studies. *Clin Pharmacol Ther*. 2011;89(1):65-74.

27. Gremmel T, Steiner S, Seidinger D, Koppensteiner R, Panzer S, Kopp CW. The influence of proton pump inhibitors on the antiplatelet potency of clopidogrel evaluated by 5 different platelet function tests. *J Cardiovasc Pharmacol*. 2010;56(5):532-9.

28. Ho PM, Maddox TM, Wang L, et al. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA*. 2009;301(9):937-44.

29. Kreutz RP, Stanek EJ, Aubert R, et al. Impact of proton pump inhibitors on the effectiveness of clopidogrel after coronary stent placement: The clopidogrel medco outcomes study. *Pharmacotherapy*. 2010;30(8):787-796.

30. Juurlink DN, Gomes T, Ko DT, et al. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ*. 2009;180(7):713-718.

31. Gaglia MA,Jr, Torguson R, Hanna N, et al. Relation of proton pump inhibitor use after percutaneous coronary intervention with drug-eluting stents to outcomes. *Am J Cardiol*. 2010;105(6):833-838.

32. Bhurke SM, Martin BC, Li C, Franks AM, Bursac Z, Said Q. Effect of the clopidogrel-proton pump inhibitor drug interaction on adverse cardiovascular events in patients with acute coronary syndrome. *Pharmacotherapy*. 2012;32(9):809-818.

33. Burkard T, Kaiser CA, Brunner-La Rocca H, et al. Combined clopidogrel and proton pump inhibitor therapy is associated with higher cardiovascular event rates after percutaneous coronary intervention: A report from the BASKET trial. *J Intern Med*. 2012;271(3):257-263.

34. Huang B, Huang Y, Li Y, et al. Adverse cardiovascular effects of concomitant use of proton pump inhibitors and clopidogrel in patients with coronary artery disease: A systematic review and meta-analysis. *Arch Med Res*. 2012;43(3):212-224.

35. Dunn SP.
Baseline proton pump inhibitor use is associated with increased cardiovascular events with and without the use of clopidogrel in the CREDO trial *Circulation*. 2008;118(A3999):815.

36. Ray WA, Murray KT, Griffin MR, et al. Outcomes with concurrent use of clopidogrel and proton-pump inhibitors: A cohort study. *Ann Intern Med*. 2010;152(6):337-345.

37. Rassen JA, Choudhry NK, Avorn J, Schneeweiss S. Cardiovascular outcomes and mortality in patients using clopidogrel with proton pump inhibitors after percutaneous coronary intervention or acute coronary syndrome. *Circulation*. 2009;120(23):2322-2329.

38. Kwok CS, Jeevanantham V, Dawn B, Loke YK. No consistent evidence of differential cardiovascular risk amongst proton-pump inhibitors when used with clopidogrel: Meta-analysis. *Int J Cardiol*. 2013;167(3):965-974.

39. Niu X, Mao L, Huang Y, et al. CYP2C19 polymorphism and clinical outcomes among patients of different races treated with clopidogrel: A systematic review and meta-analysis. *J Huazhong Univ Sci Technolog Med Sci*. 2015;35(2):147-156.

40. 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*. 2007;5(12):2429-2436.

41. Zou JJ, Xie HG, Chen SL, et al. Influence of CYP2C19 loss-of-function variants on the antiplatelet effects and cardiovascular events in clopidogrel-treated chinese patients undergoing percutaneous coronary intervention. *Eur J Clin Pharmacol*. 2013;69(4):771-777.

42. Shirai N, Furuta T, Moriyama Y, et al. Effects of CYP2C19 genotypic differences in the metabolism of omeprazole and rabeprazole on intragastric pH. *Aliment Pharmacol Ther*. 2001;15(12):1929-1937.

43. Ohbuchi M, Noguchi K, Kawamura A, Usui T. Different effects of proton pump inhibitors and famotidine on the clopidogrel metabolic activation by recombinant CYP2B6, CYP2C19 and CYP3A4. *Xenobiotica*. 2012;42(7):633-640.

44. Ogilvie BW, Yerino P, Kazmi F, et al. The proton pump inhibitor, omeprazole, but not lansoprazole or pantoprazole, is a metabolism-dependent inhibitor of CYP2C19: Implications for coadministration with clopidogrel. *Drug Metab Dispos*. 2011;39(11):2020-2033.

45. Furuta T, Iwaki T, Umemura K. Influences of different proton pump inhibitors on the anti-platelet function of clopidogrel in relation to CYP2C19 genotypes. *Br J Clin Pharmacol*. 2010;70(3):383-392.

46. Liu Q, Dang DS, Chen YF, Yan M, Shi GB, Zhao QC. The influence of omeprazole on platelet inhibition of clopidogrel in various CYP2C19 mutant alleles. *Genet Test Mol Biomarkers*. 2012;16(11):1293-1297.

47. Hokimoto S, Mizobe M, Akasaka T, et al. Impact of CYP2C19 polymorphism and proton pump inhibitors on platelet reactivity to clopidogrel and clinical outcomes following stent implantation. *Thromb Res*. 2014;133(4):599-605.

48. Liu Q, Dang DS, Chen YF, Yan M, Shi GB, Zhao QC. The influence of omeprazole on platelet inhibition of clopidogrel in various CYP2C19 mutant alleles. *Genet Test Mol Biomarkers*. 2012;16(11):1293-1297.

49. El-Halabi MM, Zgheib N, Mansour NM, et al. CYP2C19 genetic polymorphism, rabeprazole and esomeprazole have no effect on the antiplatelet action of clopidogrel. *J Cardiovasc Pharmacol*. 2013;62(1):41-49.

50. ClinicalTrials.gov. Role of CYP2C19 polymorphism in the drug interaction between clopidogrel and omeprazole
 . https://clinicaltrials.gov/ct2/show/study/NCT01094275. Updated 2016. Accessed 01/18, 2016.

51. Dean L. Omeprazole therapy and CYP2C19 genotype. http://www.ncbi.nlm.nih.gov/books/NBK100895/. Updated 2013. Accessed 01/21, 2016.

52. CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE steering committee. *Lancet*. 1996;348(9038):1329-1339.

53. Humphries TJ, Merritt GJ. Review article: Drug interactions with agents used to treat acid-related diseases. *Aliment Pharmacol Ther*. 1999;13(Suppl 3):18-26.

54. Strom CM, Goos D, Crossley B, et al. Testing for variants in CYP2C19: Population frequencies and testing experience in a clinical laboratory. *Genet Med*. 2012;14(1):95-100.

55. American Heart Association. Heart disease and stroke Statistics—2016 update . *Circulation*. 2016;133(3).

56. Reese ES, Daniel Mullins C, Beitelshees AL, Onukwugha E. Cost-effectiveness of cytochrome P450 2C19 genotype screening for selection of antiplatelet therapy with clopidogrel or prasugrel. *Pharmacotherapy*. 2012;32(4):323-332.

57. Chen XP, Tan ZR, Huang SL, Huang Z, Ou-Yang DS, Zhou HH. Isozyme-specific induction of low-dose aspirin on cytochrome P450 in healthy subjects. *Clin Pharmacol Ther*. 2003;73(3):264-271.