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Positive Clinical Response to Clopidogrel is Independent of Paraoxonase 1 Q192R and CYP2C19 Genetic Variants

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Abstract

There is increasing controversy about the influence of serum paraoxonase type 1 and cytochrome CYP2C19 in the conversion of clopidogrel to its pharmaceutically active metabolite. The effect of concomitant medication with the proton pump inhibitor omeprazole has been also subject of intense scrutiny. We present a cohort of 263 patients receiving anti-platelet aggregation treatment with clopidogrel and aspirin for 1 year. The paraoxonase 1 gene Q192R variant along with the presence of CYP2C19*2 and *3 loss of function alleles, concomitant medication with proton pump inhibitors and known cardiovascular risk factors were examined to determine their influence in disease relapse due to an ischaemic event during the 12 month treatment period.

The low number of patients suffering a relapse (20 out of 263), indicates that double anti-aggregation therapy with aspirin and clopidogrel was very effective in our patients. Among the relapsers, evidence of coronary heart disease was the most influencial factor affecting response to therapy, while the presence of the paraoxonase 1 Q192R variant, loss of function of CYP2C19, and concomitant medication with omeprazole were non-significant.

Keywords

aspirin, clopidogrel, CYP2C19, omeprazole, paraoxonase

Introduction

Platelet anti-aggregation therapy is the treatment of choice for patients with cardiovascular disease, and is achieved through the combination of aspirin, a cyclooxygenase-1 inhibitor, and a P2Y12 receptor antagonist.[1] The most commonly used P2Y12 antagonist is the thiol metabolite of clopidogrel, a pro-drug that is converted to its active form through metabolic transformation. Despite being a drug that is well tolerated, its efficacy varies among individuals due to multiple factors that reduce drug bioavailability, including interactions with other drugs and genetic background.[2,3] Indeed, there is evidence that polymorphic variants in enzymes that determine conversion of the pro-drug to its active metabolite may play an important role in determining its bioavailability. Two enzymes described as being responsible for the biotransformation of clopidogrel are the cytochrome CYP2C19,[4] and the serum paraoxonase/arylesterase type 1 (PON1).[5] While there is support for a role of CYP2C19 in the biotransformation of the drug,[6–8] the influence of the genetic variant Q192R at the PON1 gene, shown to be heavily involved in the activation of clopidogrel,[5] is currently questioned by several studies based on the analysis of both the clinical response[9–15] and platelet reactivity ex vivo.[14–18]

Besides the action of specific genetic variants, there is also controversy about the effect of concomitant medication, mainly proton pump inhibitors (PPI) and, particularly, omeprazole. As cytochrome CYP2C19 is responsible for up to 80% of omeprazole clearance,[19,20] this substrate could competitively inhibit the binding of clopidogrel to the enzyme and inhibit its biotransformation. On the other hand, there is not a conclusive evidence

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demonstrating that omeprazole affects the efficacy of clopidogrel in patients receiving both drugs depending on whether ex vivo platelet aggregation or clinical endpoints are considered.[21,22]

We have studied the influence, in disease relapse, of both the Q192R *pon1* gene variant and the most common CYP2C19 loss of function (LOF) alleles CYP2C19*2 and *3, the effect of concomitant medication with omeprazole and the presence of known cardiovascular risk factors in a cohort of 263 patients receiving combined anti-aggregation therapy with aspirin and clopidogrel for 1 year.

Methods

Subjects

All participants gave written informed consent before being included in the study, which was also approved by the clinical research ethics committee of our institution. The study included 263 caucasian patients that presented with a coronary event at 18 years of age or older and received dual platelet anti-aggregation therapy, clopidogrel (75 mg daily) and aspirin (100 mg daily), for at least 1 year after admission. None of the participants had intercurrent inflammatory disease, fever, liver failure or other known malignancies.

The in-hospital data at admission included: type of acute coronary event stable angina, unstable angina, non-ST (NSTEMI) and ST-elevation myocardial infarction (STEMI), percutaneous treatment or coronary artery bypass graft (CABG) surgery, type of stent (bare-metal or drug-eluting type) implanted in patients who underwent a percutaneous coronary intervention, analytical data at admission and pharmacological treatment at discharge (aspirin, clopidogrel, acenocoumarol, beta-adrenoreceptor antagonists, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, nitrates or statins). The follow-up data included the presentation of a coronary event or cardiac mortality.

The diagnosis of an acute coronary event at admission or in the follow up was made by clinical history, electrocardiogram and serial measurements of troponin I. Patients with NSTEMI had persistent or transient ST-segment depression or T-wave inversion, flat T waves, pseudo-normalization of T waves, or no electrocardiographic changes at presentation [23] and patients with STEMI had persistent or transient ST-segment elevation.[24] Myocardial infarction was diagnosed when troponin I serum levels were above 0.16 ng/dL. The left ventricular ejection fraction (LVEF) was calculated by echocardiography using Simpson's biplane method.[25]

Evaluation of Cardiovascular Risk Factors

The criteria that define the risk factors analyzed are explained in the legend of Table 2. After an overnight fast of at least 10 hours, blood samples were drawn for the spectrophotometric detection of serum glucose, serum creatinine, hemoglobin, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL) and total cholesterol. Troponin I levels were determined with the VIDAS troponin I Ultra assay system (bioMerieux, Marcy L'Etoil, France).

Genetic Analysis

Genomic DNA was extracted from venous blood with a commercial kit (QUIAGEN, Hilden, Germany). To detect the PON1 Q192R variant (SNP ID: rs662), 50 ng of genomic DNA were amplified by using the polymerase chain reaction (PCR) following recommendations of the polymerase manufacturer (Promega) for 35 cycles (95 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min) with the primers PON1_QR_1F (5′ to 3′) TATTGTTGCTGTGGGACCTG and PON1_QR_1R ATACTTGCCATCGGGTGAAA. After digestion of the PCR products with the restriction endonuclease *AlwI*, the R allele generated two fragments, while the Q allele did not. Likewise, for the detection of the CYP2C19*2 and CYP2C19*3 haplotypes, defined by rs4244285 and rs4986893 respectively, DNA was amplified with the primer pairs CYP2C19.2_1F: ACAACCAGAGCTTGGCATATTG, CYP2C19.2_1R: CACTGGAAGCTGCAGAACAG, and CYP2C19.3_1F: CTTTCATCCTGGGCTGTGCT, CYP2C19.3_1R: GCCTGGATGTCCATGGAGTG. The presence of wt alleles were detected upon digestion with restriction endonucleases *SmaI* and *BamHI*, which do not digest the *2 or *3 haplotypes respectively. DNA fragments were analyzed by using agarose gel electrophoresis.

Statistical Analysis

In both study groups (relapse yes or no), categorical variables were summarized as frequencies and percentages, while continuous variables as mean and standard deviation (SD) when data followed a normal distribution, or as median and interquartile (25th–75th percentile) range (IQR) when distribution departed from normality. The percentages were compared using the Chi-square (χ^2) or Fisher's exact tests as indicated (Table 2), the means by the t-test, and the medians by the Wilcoxon test. The variables that showed statistical significance in the univariate analysis were entered into a multidimensional logistic analysis. A retrospective selection of relevant variables was made based on the Akaike criteria (AIC). The obtained model was summarized in *P*-values (likelihood ratio test) and odd-ratios, which were estimated by means off 95% confidence intervals. An effect was considered to be statistically significant when the corresponding *P* value was equal or lower than 0.05. Statistical analysis was performed with the SPSS software package for Windows (IBM, NY, USA) and R (Institute for Statistics and Mathematics, Wirtschaftsuniversität Wien).

Results

Patient Inclusion and Follow up

Patients admitted to the hospital for a coronary event, under clopidogrel and aspirin treatment, were followed up during the one year treatment period to monitor relapse with a new acute coronary event of ischaemic origin as a surrogate marker for therapeutic efficacy. Coronary events at admission in this selected group were stable angina (11), unstable angina (33 patients), NSTEMI (103 patients) and STEMI (116 patients). Out of the total 263, 37 patients required re-hospitalization, due to stable or unstable coronary disease, heart failure or arrhythmia. However, relapse, defined as an acute coronary event such as unstable angina, myocardial infarction with or without ST elevation and cardiac death, was identified in 20 patients. Within this group, four patients deceased (two at our hospital), two were readmitted due to stent thrombosis, eight due to stent restenosis and the rest due to *de novo* coronary disease. No significant differences in relation to the treatment received at admission were observed between relapsers and non-relapsers (Table 1).

Factors Influencing Relapse

Among the traditional cardiovascular risk factors, presenting with diabetes mellitus (DM), a personal history of coronary disease, the number of affected arteries and the presence of coronary disease (CHD) were all significantly shown to influence disease relapse, while no other risk factors such as AHT, smoking, dyslipidemia, family history of cardiovascular disease or the type of stent implanted appeared to have an effect. Likewise, no sex differences were observed (Table 2).

Table 1. Treatment Received at Hospital Admission in Non-Relapse and Relapse Patients

Treatment	Non-Relapse	Relapse	Total
Standard[a]	162	14	176
Fibrinolysis[b]	32	2	34
Primary angioplasty[c]	26	2	28
Fibrinolysis and rescue angioplasty[d]	21	2	23
Anti-platelet therapy with glycoprotein IIb-IIIa antagonists[e]	2	0	2
Total	243	20	263

Qualitative variables are expressed as the number of patients that received each type of treatment.

[a]Standard treatment included heparin (sodium salt; 5,000 units), clopidogrel (300 mg) and aspirin (300 mg).

[b]Standard treatment plus fibrinolysis with recombinant Tissue Plasminogen Activator (administered as a 15 mg intravenous bolus, followed by 0.75 mg/kg infused over the next 30 minutes not to exceed 50 mg, and then 0.50 mg/kg over the next 60 minutes not to exceed 35 mg) or Tenecteplase (a single bolus dose as recommended by body weight, not to exceed 50 mg).

[c]Primary angioplasty was defined as the first therapy used to restore blood flow through a coronary artery suspected or known to be occluded.

[d]Rescue angioplasty refers to mechanical reopening of an occluded infarct-related artery after failed intravenous fibrinolysis.

[e]Standard treatment plus antiplatelet therapy consisting of tirofiban (0.4 mcg/kg/min for 30 minutes and then continued at 0.1 mcg/kg/min for 48 hours).

All participants were genotyped for the Q192R *PON1* gene variant (SNP ID: rs662). The allele frequency observed was 0.479 QQ (AA), 0.361 QR (A/G) and 0.160 RR (GG), similar to what is reported for this variant in similar populations. Patients were then sorted in two groups according to their *PON1* genotypes: Homozygotes for the QQ allele, previously associated with a higher risk of being non-responders, were compared against QR heterozygotes and RR homozygotes, grouped together based on their similar expected phenotypes.[5] Eleven out of 20 recurrent patients presented with the low activity QQ genotype, a higher ratio than expected (9.6), but not significant (Table 2). From the other perspective, 115 patients out of 126 with the QQ genotype did not relapse during the clopidogrel treatment period. Likewise, the distribution of patients within the QR and RR combined group did not reveal a significant genotype-phenotype association.

*CYP2C19**2 LOF haplotypes (*2A, B, C and D)[26] were detected by variation at rs4244285, showing similar allelic distributions within both groups, in the expected frequencies: 0.154 for the A allele, and 0.855 for the G allele. The *CYP2C19**3 haplotypes (A and B) were defined by variation at rs4986893, and carried by 6 heterozygotes. Patients were classified, depending on whether their carried one or two *CYP2C19* LOF alleles, in intermediate (IM) or poor (PM) metabolizers respectively. Neither intermediate nor poor metabolizers or both showed significant association with relapse (Table 2).

Concomitant medication with proton pump inhibitors was also examined. Out of 72 patients taking dual anti-aggregation therapy and omeprazole (20–40 mg daily), 8 of them relapsed with a major cardiac event (Table 2), above the expected frequency (5.7), although not significant. Similarly occurred with patients under pantoprazole treatment (20–40 mg daily) who suffered a relapse (10 patients with relapse and an expected frequency of 8.3), overall indicating that concomitant medication with any proton pump inhibitor did not pose any significant risk for relapse. Regarding treatment at discharge, no significant differences were seen between non-relapse and relapse patients, with the exception of being under acenocoumarol treatment. Finally, the type of coronary intervention, either percutaneous or CABG, did not show any significant effect.

In a retrospective multivariate analysis, using the Acaike criteria, the presence of CHD, insulin-dependent DM and being under acenocoumarol treatment, were all selected to be relevant for the prediction of relapse. Among these, the presence of CHD, and treatment with acenocoumarol both appeared as significant risk factors for relapse with a new acute coronary event (Table 3).

Table 2. Demographic, Coronary, Ventricular Function, Pharmacologic and Genotypic Data from Non–Relapse and Relapse Patients

Variable	Non—relapse (243)	Relapse (20)	*P*
Age (years)	59.6 ± 12.6	60.9 ± 12.2	> 0.05
Male, N (%)	180 (74.1)	14 (70)	> 0.05
Killip class I; N (%)[a]	214 (88.1)	17 (85.0)	> 0.05
Arterial hypertension[b]	153 (63)	16 (80)	> 0.05
Diabetes mellitus, N (%)[c]			< 0.05
No	156 (64.2)	9 (45.0)	
Non-insulin-dependent	55 (22.6)	3 (15.0)	
Insulin dependent	32 (13.2)	8 (40.0)	
Dislipidemia, N (%)[d]	119 (49)	12 (60)	> 0.05
Smoking, N (%)[e]	103 (42.4)	6 (30)	> 0.05
Family history of coronary disease, N (%)[f]	29 (11.9)	2 (10)	> 0.05
Personalhistory of coronary disease, N (%)	63 (25.9)	11 (55)	< 0.05
Hemoglobin (g/dL)	13.6 (12.0; 14.6)	12.0 (11.0; 14.5)	> 0.05
Platelet count ($x10^3$)	237 (186; 283)	253 (217; 293)	> 0.05
Serum glucose (mg/dL)	107 (94; 156)	120 (91; 178)	> 0.05
Serum creatinine (mg/dL)	1.00 (0.90; 1.24)	1.08 (0.91; 1.17)	> 0.05
Total cholesterol (mg/dL)	164 (135; 193)	175 (147; 194)	> 0.05
LDL-cholesterol (mg/dL)	95 (72; 118)	89 (77; 105)	> 0.05
Low-HDL-cholesterol, N (%)	117 (76.0)	9 (62.9)	> 0.05
Triglycerides (mg/dL)	124 (99; 165)	156 (119; 196)	> 0.05
Percutaneous CI / CABG surgery[g]	206/4 (84.8/1.6)	17/0 (85/0)	> 0.05
Two or more vessels with CHD[h]	127 (52.3)	16 (80.0)	< 0.05
Presence of CHD[i]	175/55	7/12	< 0.05
Stent type : Bare/DES/both[j]	75/106/26	4/12/1	> 0.05
Left ventricle ejection fraction (%)[k]	58 (45; 60)	60 (47; 60)	> 0.05
*PON1*QQ Genotype, N (%)	115 (47.3)	11 (55)	> 0.05
CYP2C19 IM[l]	62 (25.5)	3 (15)	> 0.05
CYP2C19 PM[m]	5 (2.0)	2 (10)	> 0.05
CYP2C19 IM + PM	67 (27.5)	5 (25)	> 0.05
Beta blockers, N (%)[n]	222 (91.4)	17 (85)	> 0.05
ACEIs, N (%)[o]	151 (62.1)	12 (60)	> 0.05
ARBs, N (%)[p]	44 (18.1)	3 (15)	> 0.05
CCB, N (%)[q]	47 (19.3)	6 (30)	> 0.05
Statins, N (%)	230 (94.6)	20 (100)	> 0.05
Acenocoumarol, N (%)	7 (3.0)	3 (15)	< 0.05
Omeprazole, N (%)[r]	64 (27.4)	8 (40)	> 0.05
Pantoprazole, N (%)[s]	96 (41.0)	10 (50)	> 0.05

Data shown are means ± SD or medians (IQR) or frecuencies (%) for non-relapsers and relapsers during the first year treatment period.

[a]Killip classification was assigned on the basis of the severity of signs of heart failure at the time of hospital admission: Killip class I was defined by the absence of rales in the lung fields; Killip class II was defined by the presence of rales in < 50% of the lung fields; Killip class In was defined by the presence of rales in > 50% of the lung fields; and Killip class IV was defined as cardiogenic shock (the presence of pulmonary oedema with hypotension -systolic blood pressure < 90 mmHg-).

[b]Arterial hypertension (AHT): when systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg, or patient receiving medication for hypertension;

[c]Diabetes mellitus: when fasting blood glucose levels > 126 mg/dL or patient treated with oral anti-diabetic agents or insulin;

[d]Dyslipidemia (DLP): if total cholesterol levels > 240 mg/dL or patient receiving lipid-lowering therapy

[e]Smoking: when the patients were active smokers at the time of the event or within a 2 year period before the event;

[f]Family history of premature coronary heart disease: when male or female first degree relatives had shown symptoms of disease before 55 or 65 years of age respectively.

[g]CABG: coronary artery bypass graft.

[h]Number of arteries affected by coronary heart disease (CHD) with >75% lumen stenosis.

[i]Presence of CHD as focal stenotic lesion or difusse narrowing. Both (h) and (i) were determined by angiography.

[j]Type of stent implanted: metal (BMS), drug-eluting (DES) or both.

[k]Left ventricular eyection fraction determined by echocardiography.

[l]IM: Intermediate metabolizers carry one, either *2 or *3, CYP2C19 loss of function allele.

[m]PM: Poor metabolizers carry 2 LOF alleles, being either *2 or *3 homozygotes, as well as *2*3 compound heterozygotes. Analyzed by Fisher's exact test.

[n]Treatment with beta adrenergic receptor antagonists.

[o]Treatment with angiotensin-converting-enzyme inhibitors.

[p]Treatment with angiotensin II receptor blockers.

[q]Treatment with calcium channel blockers.

[r]Concomitant medication with omeprazole at 20–40 mg per day.

[s]Idem with 20–40 mg pantoprazole. All pharmacological treatments shown are at discharge.

Table 3. Multidimensional Logistic Analysis

Factor	P	OR (95%CI)	AIC
Presence of CHD	.007	4.295 (1.490 ; 12.385)	126.8
Insulin dependent DM	.058	2.819 (0.966 ; 8.223)	122.7
Acenocoumarol treatment	.025	5.986 (1.246 ; 28.766)	123.5

The variables that showed statistical significance in the univariate analysis were entered into a multidimensional logistic analysis in order to define the variables that would best predict disease relapse. A retrospective selection of variables was made based on the Akaike criteria (AIC), resulting in the final selection of three variables that significantly contribute to the final disease prediction model analysis: Presence of CHD, Insulin-dependent diabetes mellitus, and treatment with acenocoumarol. The obtained model was summarized in p-values (likelihood ratio test) and odd-ratios, which were estimated by means off 95% confidence intervals. The AIC values if the corresponding variable is removed from the model are shown. For the complete set of three variables, AIC = 121.3.

Discussion

Cardiovascular disease recurrence represents a major personal, social and economic burden. Multiple factors may affect the rate of relapse in cardiovascular patients, including specific risk factors and the response to medication. In this study, we wished to focus on factors that could affect the response of our patients to the antiplatelet agent clopidogrel in combination with aspirin as antiplatelet aggregation therapy. Clinical outcome was evaluated as a surrogate marker for response to treatment since there is no clear association between platelet aggregation assays ex vivo and the occurrence of ischemic outcomes.[27,28]

The frequency of recurrent patients in this group (7.6%) was at the low end of the number of non-relapsers described in other studies, ranging from 4% to 34%.[10,12,29,30] Both relapsers and non-relapsers were equivalent from an analytical point of view, so we believe that the low incidence of new coronary events might have been favored by both a high therapeutic compliance and an elevated percentage of patients receiving additional medication at hospital discharge.

Genetic differences are thought to represent up to 80% of the individual variance to the inhibition of the ex vivo platelet aggregation by clopidogrel, an observation that has led to invest great efforts in determining the genetic factors that may affect response to medication.[3] Among these genetic factors, there is great interest in the study of the enzymes that participate in the biotransformation of clopidogrel into its active thiol metabolite. In particular, two enzymes, the cytochrome CYP2C19 and the serum arylesterase PON1 have been implicated in the activation of the pro-drug.[4–8] While there is evidence that loss of function alleles of CYP2C19 result in decreased response,[8] the role of the Q192R variant of the *pon1* gene is controversial.[9–18]

In a sample population where all three genotypes were well represented, no association was found between *pon1* genotype and relapse with an ischaemic event during the year that the patients were taking double anti-aggregation therapy with aspirin and clopidogrel. It is true that the relapse group is small, but taken from the other perspective, out of 126 patients with the QQ genotype, only 11 relapsed during that period. Power calculation estimated that the possibility to detect differences between both groups with a χ^2 test is aproximately 10% for this sample size. Even for a larger group of 100 relapsers, the possibility of finding differences between the two groups would be only 25%. Therefore, given the allelic distribution observed (47.3 vs 55.0), thousands of patients would be needed to detect a difference between the groups in their ability to respond to clopidogrel and aspirin treatment. This analysis indicates that the presence of the QQ genotype at the *pon1* gene did not represent a risk for the clinical outcome of patients taking dual anti-aggregation therapy with clopidogrel and aspirin, as suggested by other studies.[9–15]

Concomitant medication with omeprazole has been also suggested to play a role in the modulation of the response to clopidogrel. It is believed that the effect of omeprazole is exerted through mild competitive inhibition of CYP2C19, which may be responsible for up to 80% of omeprazole clearance.[19,20] Nonetheless, the role of omeprazole in the response to clopidogrel is still unclear, depending on the endpoint tested. While omeprazole treatment increases the aggregation of platelets in an ex vivo aggregation assay in response to ADP, the clinical endpoints of these patients do not show significant association with the use of a PPI.[2,20–22] Because of recent warnings, only 72 of a total of 263 of our patients received both omeprazole and clopidogrel, while the rest received other proton pump inhibitors such as pantoprazole, antacids with different mechanism of action, such as histamine 2 receptor antagonists like ranitidine or none. In this group of 72, the number of recurrent events among these (8 patients) exceeded the expected number (5.3 patients) although without reaching statistical significance. Furthermore, CYP2C19 genotyping in our patients, could not define a correlation between the presence of LOF alleles of CYP2C19 and poor response to treatment, an observation which is consistent with other cytochromes participating in the biotransformation of clopidogrel, such as CYP3A isozymes.[31] Thus, our results agree with the European Society of Cardiology (ESC) guidelines suggesting that there is no clear evidence that the pharmacokinetic interaction of clopidogrel with some proton pump inhibitors has meaningful clinical consequences. Accordingly, it appears that the benefits of avoiding or minimizing bleeding in patients at high risk outweigh the concerns raised by an unclear pharmacological interaction.[32]

In relation to traditional risk factors, it is known that age, sex, DM, AHT and dyslipidemia are included in all cardiovascular risk prediction models influencing the possibility of recurrence. The Euro Heart Survey on Diabetes and the Heart [33] and the OASIS registry [34] confirmed that patients with coronary artery disease and/or diabetes are at high risk for mortality and cardiovascular events, as seen in our study. Similarly, the GISSI-2 [35] and the GUSTO-1[36] studies found a significant higher mortality in hypertensive myocardial infarction patients when compared to normotensives, as was the rate of left ventricular failure, recurrent angina and recurrent myocardial infarction. Although there was a higher incidence of recurrent coronary events among hypertensive patients (80% vs 63% in non-relapsers), no statistical significance was observed. Likewise, dyslipidemia was also higher in the relapse group (60% vs 49%), but not significant. Power calculations of the corresponding hypothesis test for the available sample sizes for both the AHT (43.5%) and dyslipidemia (16,2%) groups reveal that larger sample sizes are required to reveal significant results (84 vs 63, and 250 vs 49, respectively). In addition, treatment of dyslipidemic patients with statins after the acute coronary event could be a protective factor for the incidence of new cardiac events, as it has been previously shown,[37] adding complexity to the interpretation of the results within this group. On the other hand, presenting with DM, the number of coronary arteries with significant lesions and the existence of coronary artery disease, the latter in agreement with previous studies,[38] proved to be significant risk factors leading to a new acute coronary event.

The type of stent has been also associated with a greater or lesser frequency of thrombosis.[39] Both bare-metal stents (BMS) and drug eluting stents (DES) induce platelet adhesion, activation and thrombus formation, so that effective anti-platelet therapy is mandatory after stent implantation. Though there is concern about the risk of late and very late stent thrombosis in patients with DES, due to delayed endothelisation, no significant influence was observed upon the recurrence of an acute coronary event between our patients with either type of stent, in agreement with others.[40]

A retrospective multivariate analysis model was used to determine the group of variables that best predict disease relapse using the Akaike information criteria, where its value is a measure of the fitness between model and data: the lower the value, the better. For the best fitting model, three variables were finally selected: presence of CHD, insulin dependent DM, and treatment with acenocoumarol. A reduced model missing any of these three variables would be a worse predictor of relapse for any set of patients, even though the *P* value for insulin dependent DM was slightly higher than 0.05, indicating that this variable was necessary for model building although not significant. Triple anti aggregation therapy with aspirin and clopidogrel plus acenocoumarol appeared as a risk factor, although we believe this is a spurious association. One of the most common clinical scenarios requiring the use of triple anticoagulant/antiplatelet therapy is the occurrence of atrial fibrillation in patients undergoing percutaneous coronary intervention or sustaining an acute coronary event. This approach clearly increases the risk of major bleeding and this is why some physicians advise against taking clopidogrel together with acenocoumarol, favoring the increased risk of suffering a new cardiovascular event.

We are aware of the limitations imposed by a small number of relapsers in this cohort, and the fact that co-administration of aspirin may rescue defects in the response to clopidogrel. However, from a clinical standpoint, we can conclude that neither the *pon1* QQ genotype nor the concomitant medication with omeprazole or loss of function of CYP2C19 appear to have a significant effect on the reappearance of new coronary events in patients receiving dual anti-platelet aggregation therapy with clopidogrel and aspirin, while the presence of CHD appeared as the most significant risk factor influencing disease relapse.

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Declaration of Conflicting Interests

The authors declare no conflicts of interest.

References

1. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Clopidogrel in unstable angina to prevent recurrent events trial investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med*. 2001;345:494–502.
2. Sweeny JM, Gorog DA, Fuster V. Antiplatelet drug 'resistance'. Part 1: mechanisms and clinical measurements. *Nat Rev Cardiol*. 2009;6:273–282.
3. 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*. 2009;302:849–857.
4. 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*. 2006;108:2244–2247.
5. Bouman HJ, Schömig E, van Werkum JW, et al. Paraoxonase-1 is a major determinant of clopidogrel efficacy. *Nat Med*. 2011;17:110–116.
6. Mega JL, Close SL, Wiviott SD, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*. 2009;360:354–362.
7. Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*. 2009;360:363–375.

8. 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*. 2010;376:1312–1319.
9. Lewis JP, Fisch AS, Ryan K, et al. Paraoxonase 1 (PON1) gene variants are not associated with clopidogrel response. *Clin Pharmacol Ther*. 2011;90:568–574.
10. Simon T, Steg PG, Becquemont L, et al. Effect of paraoxonase-1 polymorphism on clinical outcomes in patients treated with clopidogrel after an acute myocardial infarction. *Clin Pharmacol Ther*. 2011;90:561–567.
11. Hulot JS, Collet JP, Cayla G, et al. CYP2C19 but not PON1 genetic variants influence clopidogrel pharmacokinetics, pharmacodynamics, and clinical efficacy in post-myocardial infarction patients. *Circ Cardiovasc Interv*. 2011;4:422–428.
12. Delaney JT, Ramirez AH, Bowton E, et al. Predicting clopidogrel response using DNA samples linked to an electronic health record. *Clin Pharmacol Ther*. 2012;91:257–263.
13. Paré G, Ross S, Mehta SR, et al. Effect of PON1 Q192R genetic polymorphism on clopidogrel efficacy and cardiovascular events in the clopidogrel in the unstable angina to prevent recurrent events trial and the atrial fibrillation clopidogrel trial with irbesartan for prevention of vascular events. *Circ Cardiovasc Genet*. 2012;5:250–256.
14. Sibbing D, Koch W, Massberg S, et al. No association of paraoxonase-1 Q192R genotypes with platelet response to clopidogrel and risk of stent thrombosis after coronary stenting. *Eur Heart J*. 2011;32:1605–1613.
15. Trenk D, Hochholzer W, Fromm MF, et al. Paraoxonase-1 Q192R polymorphism and antiplatelet effects of clopidogrel in patients undergoing elective coronary stent placement. *Circ Cardiovasc Genet*. 2011;4:429–436.
16. Rideg O, Komócsi A, Magyarlaki T, et al. Impact of genetic variants on post-clopidogrel platelet reactivity in patients after elective percutaneous coronary intervention. *Pharmacogenomics*. 2011;12:1269–1280.
17. Tselepis AD, Tsoumani ME, Kalantzi KI, Dimitriou AA, Tellis CC, Goudevenos IA. Influence of high-density lipoprotein and paraoxonase-1 on platelet reactivity in patients with acute coronary syndromes receiving clopidogrel therapy. *J Thromb Haemost*. 2011;9:2371–2378.
18. Bonello L, Camoin-Jau L, Mancini J, et al. Factors associated with the failure of clopidogrel dose-adjustment according to platelet reactivity monitoring to optimize P2Y12-ADP receptor blockade. *Thromb Res*. 2012;130:70–74.
19. Chong E, Ensom MH. Pharmacogenetics of the proton pump inhibitors: a systematic review. *Pharmacotherapy*. 2003;23:460–471.
20. Ishizaki T, Horai Y. Review article: cytochrome P450 and the metabolism of proton pump inhibitors–emphasis on rabeprazole. *Aliment Pharmacol Ther*. 1999;13(Suppl 3):27–36.
21. O'Donoghue ML, Braunwald E, Antman EM, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: an analysis of two randomised trials. *Lancet*. 2009;374:989–997.
22. Schmidt M, Johansen MB, Robertson DJ, et al. Concomitant use of clopidogrel and proton pump inhibitors is not associated with major adverse cardiovascular events following coronary stent implantation. *Aliment Pharmacol Ther*. 2012;35:165–174.
23. Hamm CW, Bassand JP, Agewall S, et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2011;32:2999–3054.
24. Van de Werf F, Bax J, Betriu A, et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J*. 2008;29:2909–2945.
25. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards Subcommittee on Quantitation of Two-Dimensional Echocardiography. *J Am Soc Echocardiogr*. 1989;2:358–367.
26. http://www.cypalleles.ki.se/cyp2c19.htm
27. Gurbel PA. Platelet Function During Extended Prasugrel and Clopidogrel Therapy for Patients With ACS Treated Without Revascularization. The TRILOGY ACS Platelet Function Substudy: Platelet Function in TRILOGY ACS. *JAMA*. 2012;308:1785.
28. Collet JP, Cuisset T, Range G, et al. Bedside monitoring to adjust antiplatelet therapy for coronary stenting. *N Engl J Med* .. 2012;367:2100–2109.
29. Gurbel PA, Bliden KP, Guyer K, et al. Platelet reactivity in patients and recurrent events post-stenting: results of the PREPARE POST-STENTING Study. *J Am Coll Cardiol* .. 2005;46:1820–1826.
30. O'Donoghue M, Wiviott SD. Clopidogrel response variability and future therapies: clopidogrel: does one size fit all. *Circulation*. 2006;114:e600–e606.
31. Ford NF, Taubert DC. CYP2C19, and a Black Box. *J Clin Pharmacol*. 2013;53:241–248.
32. Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology (ESC), Steg PG, James SK, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur. Heart J*. 2012;33:2569–2619.
33. Lenzen M, Ryden L, Ohrvik J, et al. Diabetes known or newly detected, but not impaired glucose regulation, has a negative influence on 1-year outcome in patients with coronary artery disease: a report from the Euro Heart Survey on Diabetes and the Heart. *Eur Heart J*. 2006;27:2969–2974.
34. Malmberg K, Yusuf S, Gerstein HC, et al. Impact of diabetes on long-term prognosis in patients with unstable angina and non-Q-wave myocardial infarction: results of the OASIS Organization to Assess Strategies for Ischemic Syndromes Registry. *Circulation*. 2000;102:1014–1019.
35. Fresco C, Avanzini F, Bosi S, et al. Prognostic value of a history of hypertension in 11,483 patients with acute myocardial infarction treated with thrombolysis. GISSI-2 Investigators. Gruppo Italiano per lo Studio della, Sopravvivena nell'Infarto Miocardico. *J Hypertens*. 1996;14:743–750.
36. Aylward PE, Wilcox RG, Horgan JH, et al. Relation of increased arterial blood pressure to mortality and stroke in the context of contemporary thrombolytic therapy for acute myocardial infarction. A randomized trial. GUSTO-I Investigators. *Ann Intern Med*. 1996;125:891–900.
37. Pickin DM, McCabe CJ, Ramsay LE, et al. Cost effectiveness of HMG-CoA reductase inhibitor statin treatment related to the risk of coronary heart disease and cost of drug treatment. *Heart*. 1999;82:325–332.
38. Janardhanan R, Kenchaiah S, Velazquez EJ, et al. Extent of coronary artery disease as a predictor of outcomes in acute myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. *Am Heart J*. 2006;152:183–189.
39. Kastrati A, Dibra A, Spaulding C, et al. Meta-analysis of randomized trials on drug-eluting stents vs. bare-metal stents in patients with acute myocardial infarction. *Eur. Heart J*. 2007;28:2706–2713.
40. Kukreja N, Onuma Y, Garcia-Garcia HM, et al. The risk of stent thrombosis in patients with acute coronary syndromes treated with bare-metal and drug-eluting stents. *JACC Cardiovasc Interv*. 2009;2:534–541.