Clinical and Pharmacological Parameters Determine Relapse During Clopidogrel Treatment of Acute Coronary Syndrome

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Abstract

The therapeutic efficacy of clopidogrel as an antiplatelet drug varies among individuals, being the mainstream hypothesis that its bioavailability depends on the individual genetic background and/or interactions with other drugs. A total of 477 patients receiving double antiaggregation therapy with aspirin and clopidogrel, after suffering a first event, were followed for 1 year to record relapse, as a surrogate end point to measure their therapeutic response, as defined by presenting with an acute coronary event (unstable angina, ST-segment–elevation myocardial infarction, or non–ST-segment–elevation myocardial infarction), stent thrombosis/restenosis, or cardiac mortality. Anthropometric, clinical, and pharmacological variables along with *CYP2C19* genotypes were analyzed for their association with the disease relapse phenotype. Only 75 patients (15%) suffered a relapse, which occurred during the first 6 months of therapy, with a peak at 4.5 months. An initial univariate analysis identified that patients in the relapse group were significantly older (67.4 ± 11.0 vs 61.6 ± 12.3 years old) and presented with diffuse coronary disease, insulin-dependent type 2 diabetes mellitus dyslipidemia, and arterial hypertension. A poor clinical response to the platelet antiaggregation regime also occurred more frequently among patients taking acenocoumarol and calcium channel blockers, along with aspirin and clopidogrel, while no association was found according to *CYP2C19* genotypes. A retrospective multivariate analysis indicated that patients belonging to the nonresponder phenotype to treatment with aspirin and clopidogrel were older, presented with diffuse coronary disease, a group largely overlapping with type 2 insulin-dependent diabetes mellitus, and were taking dihidropyrimidinic calcium channel blockers.

Keywords

clopidogrel, diabetes, calcium channel blockers, CYP2C19

Clopidogrel, a noncompetitive antagonist of the P2Y12 receptor, has been widely used, in combination with aspirin, as a platelet antiaggregation regime to prevent recurrent events in patients with coronary heart disease.[1] However, its efficacy varies among individuals, leading to the concept of clopidogrel resistance. Several studies have attempted to correlate this resistance with specific genetic variants in genes that encode for enzymes proposed to mediate the biotransformation of clopidogrel. The main example is cytochrome P450 (CYP) 2C19, proposed as the key enzyme in clopidogrel's activation,[2–4] despite solid pharmacological evidence demonstrating a major role played by CYP3A isozymes in this process.[5–7] Indeed, the value of *CYP2C19* genotyping before clopidogrel administration has been the subject of intense debate, which has been presented in depth in a recent review.[8] Finally, the TAILOR-PCI study, a properly designed randomized clinical trial to estimate this issue has revealed a lack of association between *CYP2C19* genotype and the clinical response to clopidogrel.[9] The contribution of other genetic variants affecting genes such as the multidrug transporter ABCB1[10] or the serum esterase PON1,[11] have been also proposed to affect response to clopidogrel, but their contribution still remains uncertain.[12–17]

To date, there seems to be no conclusive evidence of a single or discrete number of genetic variants as responsible for diminished response to clopidogrel. A recent report predicts that a compound pharmacogenomic polygenic response score, encompassing allelic variation at multiple genes that are associated with

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increased on-treatment platelet reactivity, is able to generate a risk profile as the number of variants increases.[18] Additionally, beyond specific germinal genetic variants, recent efforts have been directed to identify epigenetic footprints that may alter the expression of genes whose products may be related or are likely to be involved in the response to clopidogrel.[19,20]

When testing pharmacokinetic and pharmacodynamic parameters of clopidogrel in young, healthy subjects, the interindividual variability seem to be unpredictable based on a single parameter, so clinical resistance is likely dependent on multiple factors beyond specific genotypes, including pharmacological interactions with other drugs and associated comorbidities.[21] Early studies have associated the use of proton pump inhibitors and calcium channel blockers (CCBs) with a reduced antiplatelet response ex vivo and decreased clinical efficacy of clopidogrel,[22,23] but there is no conclusive evidence of clinical relevance to date.[24–27] Likewise, an interaction with CYP3A statins has been suggested from ex vivo studies,[5,28] although clinical observations suggest a beneficial effect.[29,30] To this end, other drugs, such as angiotensin-converting enzyme inhibitors, have been also shown to enhance clopidogrel action, specifically increasing the risk of bleeding.[31]

Finally, comorbidities such as diabetes may play a decisive role in determining the efficacy of the dual antiplatelet regimen.[32,33]

In summary, in this complex scenario, it seems appropriate to conduct studies directed to determine those characteristics associated with a poor clinical response to clopidogrel to identify the patient profile that would benefit from alternative antiaggregant therapies.

Methods

Patients and Variables

This is the continuation of a case-control study based on a retrospective data collection, where 477 cases were recruited because of an acute coronary event and were admitted at the Cardiology Department of the Complejo Hospitalario Universitario Materno Infantil since March 2006. This study was approved by the Clinical Research Ethics Committee of our institution, and all patients gave written informed consent before joining the study. Inclusion and exclusion criteria, measurements of clinical and biochemical variables, and *CYP2C19* genotyping were performed as described previously.[17,34]

Statistical Analyses

Univariate Analysis. Categorical variables are expressed as frequencies and percentages, and continuous variables as means and standard deviations (SDs) when data followed a normal distribution, or as medians and interquartile ranges (25th-75th percentile) when distribution departed from normality. The percentages were compared, as appropriate, using the chi-square (χ^2) test or the Fisher exact test, the means by the *t*-test, and the medians by the Wilcoxon test for independent data.

Multivariate logistic analysis for relapse. The variables that showed significant association with the outcome in univariate analysis were entered into the multivariate analysis. Selection of variables based on the best subset regression and Akaike information criterion (AIC) was then performed.[35] The models were summarized as *P* values (likelihood ratio test).

Time to Relapse. A patient was considered a nonresponder to clopidogrel when any of the following events occurred: acute coronary event (unstable angina, ST-segment–elevation myocardial infarction [STEMI], or non–ST-segment–elevation myocardial infarction [NSTEMI]), stent thrombosis/restenosis, or cardiac mortality. The proportional hazard model was used to identify the factors associated with the time elapsed between the admission and the time to clopidogrel failure. A selection of variables was carried out using a stepwise method. The resulting model was summarized as *P* values and hazard rates, which were estimated by means of 95%CIs. Survival curves by the Kaplan-Meier method for the selected factors were estimated according to the levels of each factor and compared by log-rank test. Statistical significance was set at $P < .05$. The data were analyzed using the R package version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).[36]

Results

Patient Inclusion, Cardiological Characteristics, and Follow-Up

Patients admitted to the hospital for a coronary event, later discharged under double antiaggregation therapy with aspirin and clopidogrel, were invited to participate in the study, and 477 accepted. The coronary events at admission in this selected group were stable angina (46 patients), unstable angina (66 patients), NSTEMI (167 patients), and STEMI (198 patients).

Patients were followed up during the 1-year treatment period to monitor disease relapse, defined as presenting with an acute coronary event (unstable angina, STEMI, or NSTEMI), stent thrombosis/restenosis, or cardiac mortality during the 1-year treatment period with clopidogrel. These events were registered from the diagnosis present in clinical records and through telephone calls, which were also used to ascertain compliance, leading to a group of 75 patients.

An initial analysis evaluating the association of anthropometric and cardiological characteristics with

Table 1. Anthropometric and Cardiological Characteristics of the Patients at Admission and Their Influence on Disease Relapse During Treatment With Double Antiplatelet Therapy

		Relapse During Clopidogrel Treatment		
	All N = 477	No N = 402	Yes N = 75	*P* Value
Age, y	62.5 ± 12.3	61.6 ± 12.3	67.4 ± 11.0	<.001
Sex, male	348 (73.0)	296 (73.6)	52 (69.3)	.442
First event				.208
Stable angina	46 (9.6)	35 (8.7)	11 (14.7)	
Unstable angina	66 (13.8)	53 (13.2)	13 (17.3)	
NSTEMI	167 (35.0)	141 (35.1)	26 (34.7)	
STEMI	198 (41.5)	173 (43.0)	25 (33.3)	
Location				.374
Undefined	252 (52.9)	205 (51.1)	47 (62.7)	
Anterior	88 (18.5)	77 (19.2)	11 (14.7)	
Lateral	15 (3.2)	13 (3.2)	2 (2.7)	
Inferior	121 (25.4)	106 (26.4)	15 (20.0)	
Killip classification				.043
1	410 (86.0)	353 (87.8)	57 (76.0)	
2	24 (5.0)	17 (4.2)	7 (9.3)	
3	28 (5.9)	21 (5.2)	7 (9.3)	
4	15 (3.1)	11 (2.7)	4 (5.3)	
Treatment at admission[a]				.049
Standard	318 (66.7)	263 (65.4)	55 (73.3)	
Fibrinolysis	57 (11.9)	54 (13.4)	3 (4.0)	
Primary angioplasty	64 (13.4)	54 (13.4)	10 (13.3)	
Fibrinolysis + rescue angioplasty	34 (7.1)	29 (7.2)	5 (6.7)	
GPIIb/IIIa complex antagonist	4 (0.8)	2 (0.5)	2 (2.7)	
Previous stroke	41 (8.6)	34 (8.5)	7 (9.3)	.804
LVEF, %	59 (45-60)	60 (45-60)	55 (41-60)	.583
Number of stents				.980
0	71 (15.0)	61 (15.3)	10 (13.3)	
1	183 (38.7)	153 (38.4)	30 (40.0)	
2	101 (21.4)	86 (21.6)	15 (20.0)	
3	58 (12.3)	47 (11.8)	11 (14.7)	
4	37 (7.8)	31 (7.8)	6 (8.0)	
≥5	23 (4.9)	20 (5.0)	3 (4.0)	
Coronary disease[b]				.001
No	9 (1.9)	9 (2.3)	0	
Focal	336 (71.2)	298 (74.9)	38 (51.4)	
Diffuse	127 (26.9)	91 (22.9)	36 (48.6)	

LVEF, left ventricular ejection fraction; NSTEMI, non–ST-segment–elevation myocardial infarction. STEMI, ST-segment–elevation myocardial infarction.
Data shown are mean ± standard deviation, median (interquartile range), or frequency (%) for nonrelapsers and relapsers during the 1-year treatment period.
[a] Qualitative variables are expressed as the number of patients who received each type of treatment. Standard: aspirin 300 mg oral, clopidogrel 300 mg oral and intravenous heparin; fibrinolysis: tenecteplase, 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 min not to exceed 35 mg; primary coronary angioplasty (defined as the first therapy to restore blood flow through a coronary artery suspected or known to be occluded), rescue angioplasty (refers to mechanical reopening of an occluded infarct-related artery after failed intravenous fibrinolysis), or antiplatelet therapy with glycoprotein IIb/IIIa complex inhibitors (standard treatment plus antiplatelet therapy consisting of Tirofiban, 0.4 μg/kg/min for 30 min and then continued at 0.1 μg/kg/min for 48 h).
[b] Presence and extent of coronary disease as determined by angiography.

relapse revealed no significant differences in relation to age or sex at admission. Likewise, neither the type of initial event, its location and the type of intervention at admission, the Killip classification, nor the number of stents implanted significantly influenced the occurrence of a later relapse episode. Only the presence at first admission of diffuse coronary disease, defined as luminal stenosis >75%, as determined by angiography, was significantly associated with disease relapse (Table 1). The rate of relapse was higher during the first months of treatment, with a maximum around 4.5 months during the 1-year treatment period (Figure 1).

Cardiovascular Risk Factors Associated With Relapse

The evaluation of traditional cardiovascular risk factors for their association with relapse revealed that type 2 diabetes mellitus, especially the insulin-dependent type, was the most significant risk factor associated with

Table 2. Analytical Characteristics of the Patients Who Are Associated With Relapse During Treatment With Clopidogrel: Lifestyle, Comorbidities, and Analytical Variables

		Relapse During Clopidogrel Treatment		
	All N = 477	No N = 402	Yes N = 75	*P* Value
Smoker[a]				.081
Nonsmoker	187 (39.2)	156 (38.8)	31 (41.3)	
Current	194 (40.7)	171 (42.5)	23 (30.7)	
Former	96 (20.1)	75 (18.7)	21 (28.0)	
Personal history of CHD	137 (28.7)	108 (26.9)	29 (38.7)	.038
Type 2 diabetes mellitus[b]				.003
No	270 (57.8)	240 (60.8)	30 (41.7)	
Not ID	113 (24.2)	93 (23.5)	20 (27.8)	
ID	84 (18.0)	62 (15.7)	22 (30.6)	
AHT[c]	318 (66.7)	259 (64.4)	59 (78.7)	.016
Dyslipidemia[d]	264 (55.4)	211 (52.5)	53 (70.7)	.004
Blood hemoglobin (g/dL)[e]	13.2 ± 1.9	13.4 ± 1.8	12.5 ± 2.0	<.001
Platelet count ($\times 10^3/\mu L$)[f]	238.9 ± 79.6	241.1 ± 81.2	227.6 ± 70.2	.185
Leukocytes ($\times 10^3/\mu L$)[g]	9.4 ± 3.8	9.4 ± 3.8	9.2 ± 3.9	.568
Plasma glucose (mg/dL)[h]	111 (93-167)	108 (93-158)	138 (99-217)	.003
Serum creatinine (mg/dL)[i]	1.03 (0.90-1.22)	1.02 (0.90-1.20)	1.10 (0.90-1.34)	.120
LDL-cholesterol (mg/dL)[j]	90 (70-110)	92 (70-111)	86 (66-103)	.152
Triglycerides (mg/dL)[k]	128 (104-166)	129 (103-164)	124 (104-187)	.636

AHT, arterial hypertension; CHD, coronary heart disease; ID, insulin-dependent; LDL, low-density lipoprotein.
Data shown are mean ± standard deviation, median (interquartile range), or frequency (%).
[a] Active smokers at the time (current) of the event or within a 2-year period before the event (former).
[b] When fasting blood glucose is >126 mg/dL or patient is treated with oral antidiabetic agents or insulin.
[c] When systolic or diastolic blood pressure is >140 or >90 mm Hg, respectively, or taking hypotensive medication.
[d] If total cholesterol is >240 mg/dL or patient is receiving lipid-lowering therapy.
[e] Blood hemoglobin normal levels: women, 12-16 g/dL; men, 14-18 g/dL.
[f] Platelet count normal values: 150-450 $\times 10^3/\mu L$.
[g] Leukocyte count normal values: 4000-11 $\times 10^3/\mu L$.
[h] Plasma glucose normal fasting levels: 70-99 mg/dL.
[i] Serum creatinine normal values: women, 0.50-1.10 mg/dL; men, 0.70-1.30 mg/dL.
[j] Serum LDL-cholesterol: normal <129 mg/dL, borderline high, 130-159 mg/dL; high, 160-189 mg/dL; very high, >189 mg/dL.
[k] Serum triglycerides fasting values: normal, <150 mg/dL; borderline high, 150-199 mg/dL; high, 200-499 mg/dL; very high, >499 mg/dL.

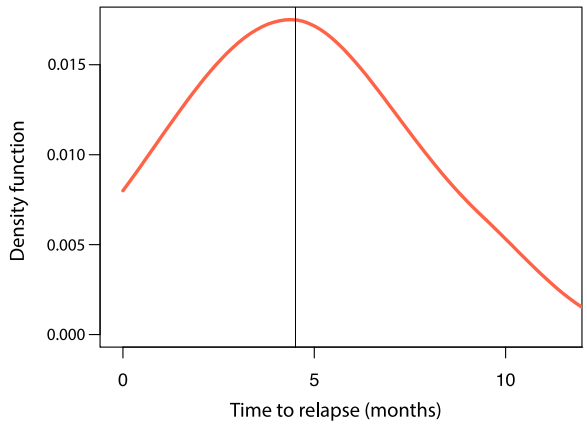

Figure 1. Rate of relapse. Curve shows the number of events per time unit during the 1-year treatment period with the platelet antiaggregation regime.

disease relapse (Table 2), with an increased probability of suffering an event during the treatment period (Figure 2A). Accordingly, higher glycemia was also significantly associated with relapse.

In addition, dyslipidemia, arterial hypertension, and a previous personal history of cardiovascular disease were also associated with a poor clinical response to the platelet antiaggregation regime in the initial univariant analysis, while smoking did not appear to be associated (Table 2). A lower hemoglobin concentration was also associated with recurrence.

Concomitant Medication and Disease Relapse

Disease relapse occurred more frequently in patients taking, along with the antiaggregation regime, acenocoumarol and CCBs, particularly among those taking drugs belonging to the dihydropyrimidinic class (DHPs) that are receiving, mostly, amlodipine (Table 3). Furthermore, patients taking CCBs had a significantly worse event-free progression than those without concomitant treatment (Figure 2B). When DHPs and nondihydropyrimidinic (NDHPs) CCBs were considered independently, only DHP CCB therapy was strongly associated with decreased survival probability,

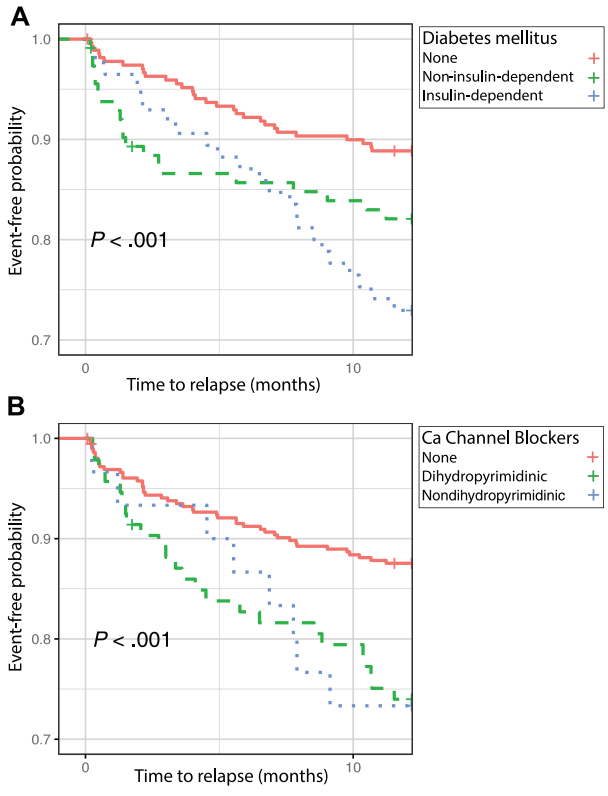

Figure 2. Kaplan-Meier survival analysis showing the event-free probability during the 12-month treatment period with antiplatelet therapy. (A) Comparison of patients with type 2 diabetes mellitus, either insulin dependent (blue) versus non–insulin dependent (green) or patients without diabetes (red). (B) Event-free probability during the time of treatment comparison between patients taking either dihydropyrimidinic CCBs (green) vs nondihydropyrimidinic CCBs or controls taking other prescriptions instead of CCBs (red). CCBs, calcium channel blockers.

while the association of NDHPs with a poorer response did not reach statistical significance.

Concomitant administration of beta-adrenergic receptor antagonists or nitrates were also associated with a poorer therapeutic response, while concomitant medication with proton pump inhibitors (omeprazole or pantoprazole), mineralocorticoid receptor antagonists, angiotensin-converting enzyme inhibitors, or angiotensin receptor blockers were not.

Association of CYP2C19 Predicted Function With Disease Relapse

Patients were genotyped for the presence of the most frequent loss-of-function (LOF) alleles for *CYP2C19*, **2*, **3*, and the gain-of-function allele **17*. Because of the low frequency of other LOF alleles for CYP2C19, the wild-type allele *1 was assumed as the default haplotype when the genotype was not *2, *3, or *17. The distribution of the *2 and *17 alleles followed the Hardy-Weinberg equilibrium ($P > .05$).

CYP2C19 function was inferred from genotypes as indicated, and no significant relationship between the CYP2C19 activity groups predicted from genotypes, and the clinical response to double antiaggregation therapy with aspirin and clopidogrel (Table 4). Patients with LOF alleles for this enzyme did not have a significantly increased risk as compared with noncarriers. Similarly, the gain-of-function genotype was not associated with a decreased risk of relapse.

In addition, there was no significant event-free probability differences between the CYP2C19 activity groups predicted from genotypes (Figure S1). In fact, patients with a predicted normal CYP2C19 metabolic activity had a worst prediction than those with a lower metabolic activity.

Multivariate Analysis of Factors Influencing Relapse

In order to identify the factors that maintain independent association with the outcome, a multivariate logistic regression analysis was performed using the AIC. Two independent models were generated as a result of data analysis. In the first model, all variables found to be associated in the univariant analysis were considered. The best-fitting model selected identified age, presenting with diffuse coronary disease, and concomitant medication, with DHP CCBs as the most significant factors defining those patients that suffered disease relapse. Removal of any of these 3 variables resulted in increased AIC values, revealing loss of fitness between model and data: The lower the value, the better the model adjusts (Table 5).

Surprisingly, type 2 diabetes and, most especially, insulin-dependent type 2 diabetes mellitus was absent from this model. We reasoned that both phenotypes, diffuse coronary disease, and type 2 diabetes may overlap, so a second model was analyzed where the variable "diffuse coronary disease" was artificially removed from the analysis. In this scenario, along with the previously selected age and use of DHP CCBs, insulin-dependent type 2 diabetes mellitus appeared as a risk factor defining the disease relapse phenotype (Table 5).

Discussion

Resistance to clopidogrel is an important issue in the management of patients who have suffered an acute coronary event, as clopidogrel is still the antiplatelet agent of choice, possibly due to its reasonable price, which may enhance patient adherence.[37] Therefore, it is of great clinical importance to identify the factors that may determine an individual's response to the double antiplatelet aggregation therapy with aspirin and clopidogrel, to define the patients that may benefit from this therapy without compromising an adverse outcome.

Table 3. Effect of Medication at Discharge on Disease Relapse

	All N = 477	Relapse		*P* Value
		No N = 402	Yes N = 75	
Beta blockers[a]	415 (87.2)	355 (88.5)	60 (80.0)	.043
ACEIs[b]	268 (56.4)	231 (57.6)	37 (50.0)	.225
ARBs[c]	107 (22.5)	90 (22.4)	17 (23.0)	.92
CCBs				.001
None	355 (74.4)	312 (77.6)	43 (57.3)	
DHP[d]	92 (19.3)	68 (16.9)	24 (32.0)	
Non-DHP[e]	30 (6.3)	22 (5.5)	8 (10.7)	
MRCA[f]	73 (15.4)	58 (14.5)	15 (20.3)	.203
Omeprazole[g]	82 (17.3)	69 (17.2)	13 (17.6)	.940
Pantoprazole[h]	161 (33.9)	136 (33.9)	25 (33.8)	.982
Acenocoumarol[i]	37 (7.8)	21 (5.2)	16 (21.6)	<.001
Nitrates	83 (17.5)	62 (15.5)	21 (28.4)	.007

ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CCBs, calcium channel blockers; DHP, dyhidropyrimidinic; MRCA, mineralocorticoid receptor antagonist.

Data shown are mean ± standard deviation, median (interquartile range), or frequency (%). In addition to a daily dose of 100 mg salicylate plus 75 mg clopidogrel, patients could also receive:

[a] Treatment with beta-adrenergic receptor antagonists: bisoprolol 2.5-10 mg/24 h, carvedilol 6.25-25 mg/12 h, and atenolol 50-100 mg/24 h.

[b] Treatment with ACEIs: ramipril 2.5-10 mg/24 h, enalapril 5-20 mg/12 h.

[c] Treatment with ARBs: losartan 12.5-100 mg/24 h.

[d] DHP compounds: amlodipine 5-10 mg/24 h, manidipine 10-20 mg/24 h, nifedipine 30-60 mg/24 h, lercanidipine 10-20 mg/24 h.

[e] Non-DHP compounds: diltiazem 90-240 mg/24 hour, verapamil 240 mg/24 h.

[f] Treatment with MCRA: spironolactone 25-100 mg/24 h or spleronone 25-50 mg/24 h.

[g] Concomitant medication with omeprazole 20-40 mg/day.

[h] Concomitant medication with pantoprazole 20-40 mg.

[i] Acenocoumarol dosage as recommended by anticoagulation international normalized ratio.

Table 4. CYP2C19 Activity Genotype and Association With Disease Relapse

	All N = 475	Relapse		*P* Value
		No N = 401	Yes N = 74	
CYP2C19 metabolic activity[a]				.320
None (*2/*2, *2/*3, *3/*3)	13 (2.7)	11 (2.7)	2 (2.7)	
Slow (wt/*2, wt/*3, *2/*17, *3/*17)	109 (22.9)	96 (23.9)	13 (17.6)	
Normal (wt/wt)	217 (45.7)	176 (43.9)	41 (55.4)	
Increased (wt/*17, *17/*17)	136 (28.6)	118 (29.4)	18 (24.3)	

Data shown are frequencies (%).

[a] *CYP2C19* genotypes were grouped based on the activity expected for each genotype. Genotypes with high expected activity included carriers of the *17 allele, either homozygous or compound heterozygous with the wild-type (wt) 1* haplotype. Homozygous carriers of the default *1 genotype represents wt activity. Slow expected activity was represented by compound heterozygotes carrying the CYP2C19*2 and CYP2C19*3 loss-of-function alleles with either the *1 wild-type or the *17 allele. On the other hand, no expected metabolic activity was represented by comprised individuals with 2 loss-of-function alleles (2*/2* and 2*/3*).

We have followed a cohort of 477 subjects receiving combined antiaggregation therapy with both aspirin and clopidogrel for 1 year and analyzed the influence of several clinical, biochemical, and genetic variables in the clinical outcome. "Resistance" to treatment was defined by presenting with an acute coronary event (unstable angina, STEMI, or NSTEMI), stent thrombosis/restenosis, or cardiac mortality during the platelet antiaggregation treatment period. This surrogate end point was chosen because (1) there is no clear association between platelet aggregation assays ex vivo and clinical response,[38–40] and (2) some of the beneficial therapeutic effects of clopidogrel may go beyond platelet aggregation inhibition.[41,42]

In this cohort of patients, disease relapse was found in 14% of the patients, closer to the lower end compared with similar studies (4% to 34%).[43–46] This good

Table 5. Logistic Multivariable Models for Disease Relapse

Model		*P* Value[a]	AIC (b)	Odds Ratio (95%CI)
1			387.0[b]	…
	Age, per y	.009	392.0[c]	1.031 (1.007-1.055)
	DHP CCBs	.090	387.9[c]	1.672 (0.932-2.998)
	Diffuse coronary disease	<.001	396.9[c]	2.574 (1.513-4.379)
2			399.1[b]	…
	Age, per y	.002	407.1[c]	1.036 (1.013-1.060)
	DHP CCBs	.060	400.7[c]	1.759 (0.988-3.132)
	Insulin-dependent T2D	.032	401.7[c]	1.927 (1.075-3.455)

AIC, Akaike information criterion; DHP CCBs, dihydropyrimidinic calcium channel blockers; T2D, type 2 diabetes.
Selection of variables was based on the best subset regression and AIC.
[a] Likelihood ratio test.
[b] AIC for the full model.
[c] AIC if the factor is removed, revealing lack of fit (increased AIC values). Note that removing any factor leads to a worse model.

response might be because of high therapeutic compliance, verbally emphasized, and an elevated percentage of patients receiving additional medication at hospital discharge. This view was supported by the rate of relapse, with most events occurring during the first months of treatment, when compliance is highest.[47]

An initial analysis was aimed at traditional risk factors. Age, sex, diabetes mellitus, arterial hypertension, dyslipidemia, and smoking were included in all cardiovascular risk prediction models influencing the possibility of recurrence. Both the OASIS registry[48] and the Euro Heart Survey on Diabetes and the Heart[49] revealed that patients with coronary artery disease and/or diabetes are at high risk of mortality and cardiovascular events. This increased risk translates to a negative response to antiplatelet therapy,[32,33] as also observed in our study. Physiologically, patients with type 2 diabetes mellitus are characterized by a prothrombotic status favored by resistance to antithrombotic physiological signals, shear-induced aggregation, nonenzymatic glycation of platelet glycoproteins, changes in the structure and conformation of platelets, increased oxidative stress, and increased platelet turnover.[50] Moreover, the diabetic state also affects profoundly the epigenetic landscape, possibly altering the expression of genes whose products alter clopidogrel pharmacokinetics and pharmacodynamics.[19,20,51]

Diabetics are, therefore, a population of patients at high risk of recurrent thrombotic events even under clopidogrel therapy.[32,33,52,53] Type 2 diabetes, especially the insulin-dependent type, was strongly associated with disease relapse in our study. This association seemed to disappear in a logistic regression analysis, where only the presence at first admission of diffuse coronary disease was significantly associated with disease relapse (Table 1). However, when we artificially removed this variable, the logistic regression model picked up insulin-dependent diabetes as the most significant variable associated with disease relapse, suggesting that both the populations of patients with diffuse coronary disease and insulin-dependent diabetes mellitus largely overlap.

Our results also showed that, in this cohort, concomitant administration of CCBs resulted in an independent increased risk of an acute event during the treatment period, and it appeared to be related to drug class, with those patients under DHP compounds displaying a heightened risk of relapse (Figure 2B). Concurrent use of CCBs with clopidogrel has been previously associated both with a decrease in the antiplatelet response ex vivo,[22,23] although this was challenged by clinical studies.[24–26]

Genetic differences are thought to represent up to 80% of the individual variance to the inhibition of the ex vivo platelet aggregation by clopidogrel and, among them, genetic variants leading to reduced CYP2C19 activity have been proposed to reduce the bioavailabilty of the active thiol metabolite.[2–4] However, we have been unable to correlate clinical response to clopidogrel with reduction or loss of CYP2C19 function, deduced from genotyping (see Figure S1), in agreement with the TAILOR-PCI study.[9]

Finally, age was also a determinant factor, as the risk increased per year of age: Patients in the relapse group were significantly older than those responding to therapy, an effect observed in other studies.[18,52]

We are aware of the limitations imposed by a small number of relapsers in this cohort, and the fact that coadministration of aspirin may rescue defects in the response to clopidogrel, as it interacts with the drug at multiple levels.[54] Furthermore, we must assume that any medication prescribed to a patient is self-administered with complete adherence to the regimen; however, we cannot know this for certain. For example, it was known if patients were on CCBs at the time of discharge, but subsequent prescription

and the adherence to CCBs therapy was not evaluated. Therefore, it is possible that some patients stopped their CCB therapy after discharge and others began treatment during the course of the 1-year follow-up period. Additionally, different adherence to other study drugs in each subgroup may have impacts on our study outcomes. Likewise, since no drug pharmacokinetic data are available, whether diabetes influences drug pharmacokinetics or pharmacodynamics or exerts its negative effects through other physiological mechanisms may not be answered.

In summary, our results show that diffuse coronary disease, likely associated with type 2 insulin-dependent diabetes mellitus, and concomitant treatment with CCBs appeared, along with age, as the most significant independent risk factors associated with the recurrence of new coronary events in patients receiving dual antiplatelet aggregation therapy with aspirin and clopidogrel. Larger prospective studies, ideally randomized controlled trials, would be required to reach a conclusion about the interaction of CCBs among these patients.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data-Sharing Statement

Access to data used in this study may be obtained through the corresponding author, upon approval from the Ethics Committee.

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