Original article

Influence of CYP2C19 Polymorphisms in Platelet Reactivity and Prognosis in an Unselected Population of Non ST Elevation Acute Coronary Syndrome

Antonio Tello-Montoliu, Eva Jover, Francisco Marín, Agustina Bernal, María L. Lozano, Beatriz Sánchez-Vega, Francisco J. Pastor, José A. Hurtado, Mariano Valdés, Vicente Vicente, and José Rivera

*Servicio de Cardiología, Hospital Universitario Virgen de la Arrixaca, El Palmar, Murcia, Spain
†Universidad de Murcia, Murcia, Spain
‡Centro Regional de Hemodonación, Murcia, Spain

ABSTRACT

Introduction and objectives: CYP2C19*2 and CYP2C19*17 alleles appear to contribute to heterogeneous clopidogrel metabolism. The aims of the present study were to assess the phenotype-genotype relationship of CYP2C19*2 and *17 allele carriage and to explore the clinical impact of those polymorphisms at 6-month follow-up of an acute event in an unselected population of non-ST elevation acute coronary syndrome.

Methods: Recruitment for the first and second objectives was 40 stable acute coronary syndrome patients under dual antiplatelet therapy at 12 months after coronary stent placement and an unselected population of 493 consecutive patients with non-ST elevation acute coronary syndrome, respectively. Platelet reactivity was assessed by optical aggregometry induced by adenosine diphosphate and thrombin receptor activating peptide, and by vasodilator-stimulated phosphorylation measurement using flow cytometry. Genotypes were determined with a TaqMan assay.

Results: Only the vasodilator-stimulated phosphoprotein phosphorylation measurement detected significant differences in on-clopidogrel platelet reactivity between the wild-type subjects and the CYP2C19*2 (P=0.20) and *17 allele carriers (P=0.048). No significant difference was found between CYP2C19*2 (HR (95%CI): 1 (0.94-1.55)], P=0.984) or *17 (HR (95%CI): 0.93 (0.61-1.43), P=0.753) allele carriage and the occurrence of adverse events at 6-month follow-up.

Conclusions: Even though CYP2C19 genotype is associated with variable on-clopidogrel platelet reactivity, it has no significant clinical influence. Prognosis of acute coronary syndromes may be influenced by a myriad of variables.

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Palabras clave:
Angina inestable
Inhibidores de la agregación plaquetaria
Plaquetas
CYP2C19

Resumen

Introducción y objetivos: Los alelos CYP2C19*2 y *17 parecen relacionarse con la variabilidad en el metabolismo del clopidogrel. Los objetivos del presente estudio son valorar la relación fenotipo-genotipo asociada a los polimorfismos CYP2C19*2 y *17 y explorar el impacto clínico de estos polimorfismos durante 6 meses de seguimiento en una población no seleccionada con síndrome coronario agudo sin elevación del segmento ST.

Métodos: Se reclutó a 40 pacientes estables doblemente antiagregados, 12 meses después del implante de stent coronario para el primer objetivo, y a 493 pacientes ingresados con síndrome coronario agudo sin elevación del segmento ST para el segundo. La reactividad plaquetaria se evaluó mediante aggregometría óptica estimulando las plaquetas con adenosina difosfato o péptido activador del receptor de trombina, y valorando la fosfoproteína estimulada por vasodilatadores fosforilada mediante citometría de flujo. El genotipo para los polimorfismos CYP2C19*2 y *17 se analizó con sondas TaqMan.

Resultados: Sólo el test de fosforilación de fosfoproteína estimulada por vasodilatadores mostró diferencias significativas en la reactividad plaquetaria entre los pacientes portadores y no portadores de los alelos CYP2C19*2 (p = 0.020) y *17 (p = 0.048). No se observó asociación con el pronóstico a 6 meses de los alelos CYP2C19*2 (HR (IC95%): 1 (0.94-1.55)], p = 0.984) y *17 (HR (IC95%): 0.93 (0.61-1.43), p = 0.753).

Palavras chave:
Angina instável
Inibidores da agregação plaquetária
Plaquetas
CYP2C19

Resumo

Introdução e objetivos: Os alelos CYP2C19*2 e *17 parecem relacionar-se com a variabilidade no metabolismo do clopidogrel. Os objetivos do presente estudo são avaliar a relação fenotipo-genotipo associado aos polimorfismos CYP2C19*2 e *17 e explorar o impacto clínico destes polimorfismos durante 6 meses de seguimento em uma população não seletiva com síndrome coronária aguda sem elevação do segmento ST.

Métodos: Foi recrutado 40 pacientes estáveis doblemente antiagregados, 12 meses após a implantação de stent coronariano para o primeiro objetivo, e 493 pacientes internados com síndrome coronariana aguda sem elevação do segmento ST para o segundo. A reatividade plaquetária foi avaliada mediante agregometria óptica estimulando as plaquetas com adenosina difosfato ou peptídeo ativador do receptor de trombina, e avaliando a fosfoproteína estimulada por vasodilatadores fosforilados mediante citometria de fluxo. O genotipo para os polimorfismos CYP2C19*2 e *17 foi analisado com sondas TaqMan.

Resultados: Sólo o teste de fosforilação de fosfoproteína estimulada por vasodilatadores mostrou diferenças significativas na reatividade plaquetária entre os pacientes portadores e não portadores dos alelos CYP2C19*2 (p = 0.020) e *17 (p = 0.048). Não foi observada associação com o pronóstico a 6 meses dos alelos CYP2C19*2 ([IC95%]: 1 (0.94-1.55)], p = 0.984) e *17 ([IC95%]: 0.93 (0.61-1.43], p = 0.753).
CONCLUSIONS: Los polimorfismos de CYPC219 *2 y *17 se relacionaron con la reactividad plaquetaria, pero no con el pronóstico a 6 meses, en el que multitud de variables deben influir.

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Platelet Function Assays

Platelet Aggregation Assays

Light transmittance aggregometry was performed using a standard aggregometer (Aggrecorder II, Menarini Diagnostics, Florence, Italy) set at 37 °C and 1000 rpm. Changes in light transmission were monitored for 5 min in unadjusted platelet-rich plasma after stimulating with 5 μM and 10 μM ADP (DiaMed, Cressier, Switzerland) and 25 μM thrombin receptor activating peptide (TRAP) (Sigma-Aldrich Inc, St Louis, Missouri, United States). The endpoint of platelet aggregation corresponds to percentage of maximal change in light transmission, considering the autologous platelet-poor plasma as 100%. The TRAP-inducible aggregation response was used as an intra-assay positive control for each patient, as this response is mediated by the thrombin receptor protease-activating receptor-1 and is not blocked by thienopyridines.

Platelet Reactivity Index-Vasodilator Stimulated Phosphoprotein Assay

The measurement of the vasodilator-stimulated phosphoprotein (VASP) phosphorylation levels is a marker of P2Y12 reactivity and thus of clopidogrel-induced inhibition.11 Phosphorylated VASP levels were quantified through assessment of the platelet reactivity index (PRI) by flow cytometry (Beckman Coulter FC500, Miami, Florida, United States) using labeled monoclonal antibodies and following a standardized assay (Platelet VASP, Diagnostica Stago, Biocytex Inc, Marseille, France) according to manufacturer’s instructions. Platelet population was identified on forward and size scatter distributions and 5000 events were gated. The extent of VASP phosphorylation was assessed by difference in the geometric mean fluorescence intensity (MFI) values in samples incubated with PGE1 in the presence or absence of ADP (MFI_{PGE1+ADP}, and MFI_{PGE1}, respectively). After subtraction of negative isotypic control values from the corresponding fluorescence values, PRI(%) was calculated according to the following formula: PRI(%)=\frac{(MFI_{PGE1+ADP})-MFI_{PGE1}}{MFI_{PGE1}}\times100. The ratio was expressed as mean percentage platelet reactivity, and inversely correlated with the clopidogrel-mediated platelet inhibition.

Genotyping

Genomic DNA was purified using the Puragene Blood Core Kit B (QIAGEN Sciences, Germantown, Maryland, United States). Genotype analysis of the CYP2C19*2 (681G>A; rs4244285) and CYP2C19*17 (–806C>T; rs12248560) polymorphisms was performed with TaqMan SNP Genotyping Assays (Applied Biosystems, Carlsbad, California, United States) following the manufacturer’s instructions, using LC480 Real Time PCR (Roche, Basel, Switzerland). Genotypes were blind-analyzed for the patient’s platelet aggregation values and clinical outcome. To control for correct sample handling, genotyping was repeated in 20% of the patients, providing identical results. All patients included in the study were genotyped for both allele variants; thus the “call rate” was 100%.

Statistical Analysis

Variables are presented as mean±standard deviation, counts (percentages), or median (interquartile range). Kolmogorov-Smirnov test was used to check for normal distribution of continuous data. Categorical variables were compared using χ² test. Comparisons among continuous variables were assessed using two-sided unpaired t-student or Mann Whitney U tests depending on the data distribution. Calculations for endpoints of interest were based on the comparison of the CYP2C19*2 and CYP2C19*17 allele carriage status among wild-type subjects (G/G or C/C, respectively) and carriers of the polymorphic allele (combination of both G/A and A/A, and C/T and T/T, respectively). A dominant model was chosen in both cases because the polymorphic *A or *T alleles result in a complete loss or gain of enzyme function, which means that one mutant allele exerts a relevant effect on overall enzyme activity. Deviation from the Hardy-Weinberg equilibrium was assessed in both populations using χ² test for each polymorphism. Unadjusted Cox regression proportional hazards model was used to compare outcomes throughout the follow-up period between polymorphic and wild-type allele status, with the wild-type being the reference group in each case. The overall free-survival rates were calculated using Kaplan-Meier method, and the differences determined using the log rank test. All P-values <.05 were accepted as statistically significant. Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, Illinois, United States).

RESULTS

Relationship Between CYP2C19 Genotype and On-Treatment Platelet Reactivity

Baseline clinical characteristics of the 40 patients assessed for on-treatment platelet reactivity are shown in Table 1. Briefly, the mean age in this population was 65.8±10 years; 36 (90%) were males. All patients had stent placement, 32 (80%) of them drug-eluting and 8 (20%) bare-metal stents. The genotype distribution was 77.5% GG, 17.5% GA, and 5% AA for the CYP2C19*2 polymorphism and 67.5% CC, 30% CT, and 2.5% TT for the CYP2C19*17 polymorphism. For the CYP2C19*17 genotype distributions, no significant deviation from the Hardy-Weinberg equilibrium was observed (P=.698), unlike CYP2C19*2 (P=.009). On-treatment platelet reactivity in patients according to CYP2C19*2 and CYP2C19*17 polymorphisms is summarized in Table 2. As shown, nonsignificant differences were observed between wild-type and polymorphic subjects in the residual platelet reactivity induced by 5 and 10 μM ADP measured by light transmittance aggregometry. The TRAP-induced aggregation measured by light transmittance aggregometry was in the normal range for healthy subjects among wild-type and polymorphic patients (data not shown).

On the other hand, CYP2C19*2 *A allele carriers displayed higher platelet reactivity in the PRI-VASP than wild-type patients (G/G) (72.3±11.9% vs 56.1±18.8%, P=.02) (Fig. 1A). Likewise, lower PRI-VASP was significantly found among CYP2C19*17 *T allele carriers compared with wild-type (C/C) patients (51.3±17.8% vs 63.8±18%, P=.048) (Fig. 1B).

We analyzed patients at high risk of thrombosis according to the cut-off level for on-clopidogrel platelet reactivity in the setting of PCI proposed in a recent consensus.12 In our population, 26 (65%) and 29 (72.5%) patients displayed high on-treatment platelet reactivity as measured by ADP-induced aggregation and PRI-VASP, respectively. Despite the low number of patients evaluated, both methods used to test residual platelet reactivity showed a trend for higher representation of patients with increased on-clopidogrel platelet reactivity among carriers of the CYP2C19*2 *A allele, whereas carriage of the CYP2C19*17 *T allele did the opposite (Table 3).
Table 1
Baseline Characteristics of 40 Non-ST-Segment Elevation Acute Coronary Syndrome Patients Included in 1st Objective

<table>
<thead>
<tr>
<th>Variable</th>
<th>CYP2C19*2 allele</th>
<th>CYP2C19*17 allele</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no. (%)</td>
<td>G/G</td>
<td>*/A</td>
<td>C/C</td>
</tr>
<tr>
<td></td>
<td>31 (77.5)</td>
<td>9 (22.5)</td>
<td>27 (67.5)</td>
</tr>
<tr>
<td>Age, years</td>
<td>65.9±8.9</td>
<td>65.8±13.7</td>
<td>66.5±10.7</td>
</tr>
<tr>
<td>Age ≥65 years, %</td>
<td>54.8</td>
<td>66.7</td>
<td>63</td>
</tr>
<tr>
<td>Sex (male), %</td>
<td>93.5</td>
<td>77.8</td>
<td>85.2</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>78.6</td>
<td>66.7</td>
<td>68</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>64.3</td>
<td>44.4</td>
<td>52</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>50</td>
<td>33.3</td>
<td>44</td>
</tr>
<tr>
<td>Smoking habit, %</td>
<td>25</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>BMS, %</td>
<td>22.6</td>
<td>11.1</td>
<td>22.2</td>
</tr>
<tr>
<td>DES, %</td>
<td>77.4</td>
<td>88.9</td>
<td>77.8</td>
</tr>
</tbody>
</table>

BMS, bare-metal stent; DES, drug-eluting stent.
Age is expressed as mean±standard deviation.

Second Objective. Prognostic Significance of the CYP2C19*2 */A and *17 */T Polymorphisms

Because of early discontinuation of clopidogrel therapy in 65 included cases, 428 patients were finally recommended by their cardiologist and assessed in our study. Demographic and basal characteristics of the 428 patients are detailed in Table 4. The mean age was 67.3±12.2 years in the cohort of 278 (65%) males and 150 (35%) females. Concerning nSTE-ACS presentation, 39.6% of enrolled patients presented down-looping ST segment and 59.3% showed increased Troponin-T levels (above standard cut-off point). Mean TIMI risk score of the entire cohort was 3.22±1.54. The genotype distribution was 71.96% G/G, 24.77% G/A, and 3.27% A/A for the CYP2C19*2 polymorphism, and 66.12% C/C, 30.14% C/T, and 3.74% T/T for the CYP2C19*17. For genotype distribution, no significant deviation from the Hardy-Weinberg equilibrium was observed (P=0.527 and P=0.905, respectively). Thus, we found 120 patients with the presence of the CYP2C19*2 */A allele (28%) in our population, and 145 CYP2C19*17 */T allele carriers (33.9%) (Table 4). As shown, there were nonsignificant differences (P>0.05) in baseline and demographic characteristics, medications, and management of patients irrespective of genotype (Table 4), with the exception of significant differences in down-looping of ST segment among CYP2C19*17 allele carriers (P=0.05) as well as catheterization (without statistical significance in stent placement rates) and nitrate prescription frequencies (P=0.034 and 0.037, respectively).

Table 2
Effect of the CYP2C19 Genotype in On-Clopidogrel Adenosine Diphosphate-Induced Maximal Platelet Aggregation in Patients With Elective Percutaneous Coronary Intervention and Stent Implantation

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>No.</th>
<th>Platelet aggregation, ADP 5 μM</th>
<th>Platelet aggregation, ADP 10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>31</td>
<td>47.1±14.3</td>
<td>54.2±15.5</td>
</tr>
<tr>
<td>*/A</td>
<td>9</td>
<td>54.2±12.5</td>
<td>62.6±17.4</td>
</tr>
<tr>
<td>P-value</td>
<td>.190</td>
<td>.190</td>
<td></td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>27</td>
<td>50.3±14.6</td>
<td>58.1±16.4</td>
</tr>
<tr>
<td>*/T</td>
<td>13</td>
<td>45.5±12.8</td>
<td>51.9±15.4</td>
</tr>
<tr>
<td>P-value</td>
<td>.319</td>
<td>.265</td>
<td></td>
</tr>
</tbody>
</table>

ADP, adenosine diphosphate.
Results are shown as mean values of maximal percentage of aggregation, mean±standard deviation.

Figure 1. Effect of the CYP2C19 genotype on platelet reactivity index-vasodilator-stimulated phosphoprotein assay on patients that underwent elective percutaneous coronary intervention and stent implantation. A, effect of the CYP2C19*2 allele variant. B, effect of the CYP2C19*17 allele variant. Vasodilator-stimulated phosphoprotein phosphorylation levels were evaluated by flow cytometry in citrated whole blood by mean of a commercially available assay as described in “Methods”. Boxes describe interquartile range. Solid line is median, and lower and upper limits denote the 2nd and 98th percentiles. PRI-VASP, platelet reactivity index-vasodilators-stimulated phosphoprotein.
Table 3
Effect of CYP2C19*2 and CYP2C19*17 Genotype on the Prevalence of High-Risk Patients According to High On-Clopidogrel Platelet Reactivity

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>CYP2C19*2</th>
<th>CYP2C19*17</th>
<th>LTA-ADP (5 μM) ≥46%, no. (%)</th>
<th>PRI-VASP ≥50%, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>31 (77.5)</td>
<td>18 (58.1)</td>
<td>20 (64.5)</td>
<td></td>
</tr>
<tr>
<td>*A</td>
<td>9 (22.5)</td>
<td>8 (88.9)</td>
<td>9 (100)</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>.091</td>
<td>.036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>19 (47.5)</td>
<td>13 (68.4)</td>
<td>14 (73.7)</td>
<td></td>
</tr>
<tr>
<td>*A</td>
<td>8 (20)</td>
<td>7 (87.5)</td>
<td>8 (100)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>12 (30)</td>
<td>5 (41.7)</td>
<td>6 (50)</td>
<td></td>
</tr>
<tr>
<td>*A</td>
<td>1 (2.5)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26 (65)</td>
<td>29 (72.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LTA-ADP, maximal platelet aggregation induced with adenosine diphosphate; PRI-VASP, platelet reactivity index-vasodilator-stimulated phosphoprotein.

The effects of each polymorphic allele carriage on platelet function tests has been assessed in two ways: A) individual effect of each polymorphism, and B) combination of both polymorphisms.

Data are shown as number of cases (percentage of cases). Patients included in 1st objective were classified as "high-risk patients" when maximal aggregation induced by ADP 5 μM was ≥46%, or when PRI-VASP values were ≥50% according to cut-offs established by Bonello et al. in a recent consensus. Prevalence of high-risk patients among carriers and noncarriers of the variant alleles were compared by using χ² test.

Complete 6-month follow-up data were available in 412 (96.3%) of the 428 patients assessed. Among those, 97 (23.5%) patients had adverse events in this follow-up period.

In univariate Cox regression analysis (Table 5), we found that CYP2C19*2 loss-of-function allele (*A) carriage was not a significant predictor of adverse outcomes ([HR (95%CI): 0.99 (0.64-1.55)], P=0.984). Moreover, gain-of-function allele CYP2C19*17 (*T) was not significantly associated with lower endpoint rates at 6-month follow-up ([HR (95%CI): 0.75 (0.61-1.43)], P=0.753). As illustrated in Figure 2, cumulative survival at

Table 4
Baseline Characteristics of Patients With Non-ST-Segment Elevation Acute Coronary Syndrome Enrolled to Perform the 2nd Objective, According to CYP2C19 Genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>CYP2C19*2</th>
<th>CYP2C19*17</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G/G</td>
<td>*A</td>
<td>C/C</td>
</tr>
<tr>
<td>Patients, no. (%)</td>
<td>308 (72)</td>
<td>120 (28)</td>
<td>283 (66.1)</td>
</tr>
<tr>
<td>Age, years</td>
<td>67.3±12</td>
<td>67.3±12.8</td>
<td>67.3±12.2</td>
</tr>
<tr>
<td>Age ≥65 years, %</td>
<td>65.6</td>
<td>58.3</td>
<td>65</td>
</tr>
<tr>
<td>Sex (male), %</td>
<td>64.3</td>
<td>66.7</td>
<td>66.4</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>68.1</td>
<td>75</td>
<td>68.8</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>55.2</td>
<td>53.3</td>
<td>54.8</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>42.7</td>
<td>41.7</td>
<td>40.1</td>
</tr>
<tr>
<td>Smoking habit, %</td>
<td>22.4</td>
<td>21</td>
<td>23.4</td>
</tr>
<tr>
<td>At least 3 CVRF, %</td>
<td>39.2</td>
<td>40</td>
<td>37.7</td>
</tr>
<tr>
<td>ST segment descent, %</td>
<td>37.8</td>
<td>44.2</td>
<td>42.9</td>
</tr>
<tr>
<td>Troponin T ≥0.1 ng/mL, %</td>
<td>59.2</td>
<td>59.8</td>
<td>60.6</td>
</tr>
<tr>
<td>Significant lesion, %</td>
<td>30.2</td>
<td>31.3</td>
<td>29.9</td>
</tr>
<tr>
<td>Symptoms of severe angina, %</td>
<td>29.4</td>
<td>37.4</td>
<td>29.2</td>
</tr>
<tr>
<td>TIMI score</td>
<td>3.2±1.5</td>
<td>3.4±1.6</td>
<td>3.2±1.5</td>
</tr>
</tbody>
</table>

Management during hospitalization
- Catheterization: 71.3, 75.2, 75.7, 66, 72.4
- With stent: 46, 44.6, 48.3, 40.7, 45.6
- Without stent: 25.3, 30.6, 27.4, 25.3, 26.8
- CABG: 10.1, 14.4, 11.7, 10.4, 11.2

Treatment during 6-month follow-up period
- ASA: 96.4, 95.8, 95.4, 97.9, 96.3
- Clopidogrel: 100, 100, 100, 100, 100
- β-blockers: 86.8, 90.9, 90, 83.3, 87.9
- ACEI: 53.3, 50, 56.9, 46.9, 53.7
- Nitrate: 28.1, 39.6, 36, 21, 31
- ARB: 14.2, 22.7, 18, 13.5, 16.6
- CCB: 20.1, 20.4, 22.3, 15.6, 20.2
- Statin: 89.5, 90.9, 92, 85.4, 89.9

ACEI, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; ASA, acetylsalicylic acid; CABG: coronary artery by-pass grafting; CCB, calcium channel blockers; CVRF, cardiovascular risk factors; significant lesion, coronary stenosis ≥50%.

Age and TIMI score expressed as mean±standard deviation.
this short follow-up period was not significantly influenced by the 
CYP2C19 genotype, according to the univariate analysis (Table 5).
Conversely, several of the risk factors used to calculate the TIMI 
risk score showed statistically significant impact on clinical 
outcome in the univariate analysis, such as age ≥65 years, 
electrocardiographic abnormalities with ST segment descent, and 
symptoms of severe angina (Table 5). Indeed, the TIMI risk score 
was a potent predictor of adverse clinical outcomes in the whole 
population (HR (95%CI): 1.41 (1.23–1.62), P <.001). Additionally, 
female sex was significantly associated with adverse clinical 
outcome (HR (95%CI): 1.62 (1.09–2.42), P =.018). Nonetheless, in 
multivariate analysis only age ≥65 years and electrocardiographic 
abnormalities with ST segment descent remained significantly 
associated with adverse clinical outcomes at 6-month follow-up 
(P =.001, and P =.028, respectively) (Table 5). A trend towards poor 
outcome was shown for symptoms of severe angina during the 
preceding 24 h (P =.051).

**DISCUSSION**

Discrepancies reported in on-clopidogrel platelet reactivity 
and the impact of both polymorphisms, CYP2C19*2 and *17, may be 
related to the number of patients tested, but also to the fact that 
the PRI-VASP test specifically explores the P2Y12 receptor function and 
seems to be more sensitive to test biological effects of clopidogrel 
according to the CYP2C19 status (Table 3), unlike the P2Y12 receptors 
driving ADP-induced platelet aggregation13 by optical aggregometry. In addition, the poor correlation between the various 
platelet function tests14,15 appears to vary according to the underlying influencing factors for high on-treatment platelet reactivity.16

Several trials have found an association of CYP2C19*2 and poor 
clinical prognosis.3,4,17 Our data suggest that the clinical impact of 
a single polymorphism may have been reduced in these moderate-
risk patients because fewer than 50% of them underwent PCI7,18 for 
stent placement.18 Other studies have found that ischemic events 
and stent thrombosis occurred early after starting clopidogrel 
treatment in association with PCI and stent placement procedures.3,4,18,19 Patients undergoing PCI may have a different status 
of platelet reactivity.20 Indeed, several studies have found positive

**Figure 2.** Kaplan Meier curves for CYP2C19*2, and *17 allele carriers and non-
carriers followed up during 6 months. A, cumulative survival for CYP2C19*2 
allele carriers (GA+AA, discontinuous line) and noncarriers (GG, continuous 
line). B, cumulative survival for CYP2C19*17 allele carriers (CT+TT, 
discontinuous line) and noncarriers (CC, continuous line).

results when more than 70% of patients underwent PCI3–5,19 irrespective of the small sample size.2,19 On the contrary, in a 
genetic analysis of the Clopidogrel in Unstable Angina to prevent 
Recurrent Events (CURE)7 trial, when lower PCI frequencies were 
assessed (18% PCI, 15.5% with stent), CYP2C19*2 polymorphic 
allele status (*2/*2, *2/*3 and *3/*3) was not associated with 
adverse events.

Diabetes has been consistently associated with platelet 
hyperreactivity, and together with CYP2C19*2 polymorphism 
constitutes the best discriminating factor to explain insufficient 
response to clopidogrel.2,4,5 Nonetheless, results from a recent meta-
analysis in which 91% of the patients had undergone PCI found that 
the impact of CYP2C19*2 allele status was more associated with 
high risk of adverse clinical outcomes than other clinical factors.3 
According to those findings, diabetes was more frequent (42.4%) in 
our population than others (23%-28%).5,18 However, higher 
diabetes prevalence in our population is apparently not sufficient 
to significantly increase the risk of *2 allele carriers, even when 
combined with a PCI frequency of nearly 70% or a stent 
implantation rate of almost 50%. Therefore, PCI and stent 
placement may be considered as high risk factors, more so than 
other clinical factors, in CYP2C19*2 allele carriers under dual 
antiplatelet therapy. Accordingly, “high risk populations” must be 
defined with care depending on the underlying clinical scenarios. 
Even then, CYP2C19*2 allele status only explains 5% to 15.2% of the 
hesitaneous response to clopidogrel.2

**Table 5**

Cox Regression Analysis for the Endpoint Events (Yes/No) Followed up During 
6 Months According to Genotype and TIMI Risk Score Factors (2nd Objective)

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95%CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*2; *1(A vs G)</td>
<td>1.0 (0.94–1.55)</td>
<td>.984</td>
</tr>
<tr>
<td>CYP2C19*17; *1T vs C/C</td>
<td>0.93 (0.51–1.43)</td>
<td>.753</td>
</tr>
<tr>
<td>Female sex</td>
<td>1.62 (1.09–2.42)</td>
<td>.018</td>
</tr>
<tr>
<td>Age ≥65 years</td>
<td>2.45 (1.50–4.01)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cardiovascular risk factors ≥3</td>
<td>1.28 (0.86–1.91)</td>
<td>.225</td>
</tr>
<tr>
<td>Coronary stenosis ≥50%</td>
<td>1.40 (0.91–2.10)</td>
<td>.132</td>
</tr>
<tr>
<td>ST segment depression</td>
<td>1.68 (1.13–2.51)</td>
<td>.011</td>
</tr>
<tr>
<td>Previous ASA</td>
<td>1.40 (0.80–2.10)</td>
<td>.113</td>
</tr>
<tr>
<td>Symptoms of severe angina</td>
<td>1.60 (1.04–2.50)</td>
<td>.034</td>
</tr>
<tr>
<td>Troponin T ≥0.1ng/mL</td>
<td>1.34 (0.87–2.05)</td>
<td>.182</td>
</tr>
<tr>
<td>TIMI risk score</td>
<td>1.41 (1.23–1.62)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Multivariate analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female sex</td>
<td>1.29 (0.82–2.02)</td>
<td>.272</td>
</tr>
<tr>
<td>Age ≥65 years</td>
<td>2.52 (1.44–4.42)</td>
<td>.001</td>
</tr>
<tr>
<td>Coronary stenosis ≥50%</td>
<td>1.47 (0.88–2.46)</td>
<td>.138</td>
</tr>
<tr>
<td>ST segment depression</td>
<td>1.63 (1.05–2.53)</td>
<td>.028</td>
</tr>
<tr>
<td>Previous ASA</td>
<td>1.10 (0.66–1.82)</td>
<td>.722</td>
</tr>
<tr>
<td>Symptoms of severe angina</td>
<td>1.56 (1.24–2.92)</td>
<td>.051</td>
</tr>
</tbody>
</table>

ASA, acetylsalicylic acid; 95%CI, 95% confidence interval; HR, hazard ratio.

The reference group used to calculate the interaction between underlying allele 
status for *2 and *17 in the univariate analysis was the wild-type allele variant.
On the other hand, the clinical significance of CYP2C19*17 allele carriage remains to be fully elucidated. Some studies have related this polymorphism with greater response to clopidogrel and an increased risk of bleeding. There is little agreement about a neutral or protective effect on ischemic events. Our results failed to find a significant interaction between *17 allele status and adverse events, even when lower rates of catheterization were reported in *17 allele carriers than noncarriers (66% vs 75.7%, P=.034), probably due to our moderate-risk population. Positive studies associating CYP2C19*17 with a reduction of adverse events are based on a reduced number of patients undergoing PCI (18%) and stent deployment (15.5%). Nevertheless, higher PCI rates (90-100%) are associated with nonreduction in ischemic endpoints, irrespective of CYP2C19*17 allele status. The PLATO trial might be comparable with ours, given the PCI frequencies (66%), but it provides information only about the association between *17 allele status and bleeding risk. Thus, in patients with moderate to higher thrombotic risk the “protective” clinical impact of CYP2C19*17 may be lower. Unfortunately, we did not record bleeding rates in our patients to explore the influence of these polymorphisms in possible hemorrhagic complications in our particular cohort.

The high interindividual variability of the response to clopidogrel remains a clinically relevant issue. Even though such heterogeneity has been shown to be a multifaceted process, several studies have suggested a link among CYP2C19 genotype and prognostic implication. However, female sex (35%) was consistent with adverse clinical outcomes, in concordance with results from Mega et al. The individual prognostic relevance may depend on the clinical setting. Demographic and basal characteristics, together with CYP2C19 status, in our cohort (42.2% diabetes, 35% female sex, and 45.6% stent placement) may explain, at least in part, the trend (albeit nonsignificant) of the Kaplan Meier survival curves. A phenotype results from the combination of genotype and the environmental influence that may be the most important variable in cardiovascular disease.

**Limitations**

First, we found a high incidence of high-residual platelet reactivity among first objective patients, which may be a limitation to achieve statistical differences according to genotype in platelet function testing by using light transmission aggregometry. We do not find a categorical explanation for these results. However, a higher rate of DES use related with defective neointimal healing in coronary arteries may be related with higher platelet reactivity. Second, sample size in both populations was limited and was based on the maximum number of patients consenting to participate. We cannot exclude that it was underpowered, as in previous reports. The allelic prevalence was very similar to other studies, with 3.3% and 3.7% of A/A (CYP2C19*2) and T/T (CYP2C19*17), respectively. Moreover, we assessed entirely Caucasian-based populations; thus our results might be specific to our patient populations and the way they were managed. Therefore, our study might underestimate the influence of genetic polymorphisms on the effects of clopidogrel in our particular settings. Moreover, major studies assessing the impact of CYP2C19 had longer follow-up periods; we performed a relatively short 6-month follow-up, as previously.

We studied only the two most common polymorphisms associated with clinical outcomes; however, other alleles or baseline/demographic factors may be relevant for individual patients. Another limitation of the 2nd objective study is the short follow-up period. The lack of assessing clopidogrel compliance in the 2nd objective population also may be a limitation. Finally, the use of two different populations precludes establishing associations between on-treatment platelet reactivity, cardiovascular outcomes, and CYP2C19 allele status.

**CONCLUSIONS**

The CYP2C19*2 *A and CYP2C19*17 *T allele variants are significantly associated with increased and diminished on-clopidogrel platelet reactivity, respectively, when the specific effect of clopidogrel on the P2Y12 is tested.

However, CYP2C19 status has inconsistent influence on adverse events in unselected nSTE-ACS patients at moderate risk within a 6-month follow-up. Such populations may be more impacted by other clinical/demographic factors. A different impact may exist in clopidogrel benefit with respect to preventing adverse events such as stent thrombosis or recurrence of acute coronary syndrome.

Arterial thrombosis might be influenced by a myriad of variables. Findings obtained by any trial should be interpreted with caution depending on the different clinical settings.

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**CONFLICT OF INTEREST**

None declared.

**REFERENCES**


