CYP2C19 (+ or −)*2/(+ or −)*17 Diplotypes: Prognostic impactson patients with acute coronary syndrome

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ABSTRACT

Purpose: To investigate the prognostic impacts of a combined clopidogrel-met abolizing genotypes CYP2C19 (+ or −)*2/(+ or −)*17 on patients with acute coronary syndrome (ACS). Population and methods: Prospective, longitudinal study of 95 patients admitted for ACS to a single coronary care unit. Patients less than 75 years of age, who survived hospitalization and to whom clopidogrel was prescribed, were included. CYP2C19 genotyping was performed at discharge. For analysis, the patients were grouped as follows: Group A ([+]*2/[+]*17) n = 8; Group B ([+]*2/[-]*17) n = 18; Group C ([-]*2/[+]*17) n = 27; and Group D ([-]*2/[−]*17) n = 42. Platelet function was assessed by an ADP platelet aggregation test using a commercially available kit. The primary endpoint was a composite of mortality or readmission for ACS. The median time of follow-up was 136.0 (79.0 – 188.0) days. Results: The mean age of the study patients was 59.9 ± 10.7 years, and 83.2% were male. The allele frequencies of CYP2C19*2 and CYP2C19*17 were 14.2% and 20%, respectively. Both allele frequencies were in Hardy Weinberg equilibrium. The patient groups were homogenous for demographic data, cardiovascular risk factors, GRACE and CRUSADE bleeding scores, left ventricular ejection fraction, and coronary anatomy. ADP platelet aggregation was similar for all groups (respective rates for groups A, B, C, D were 17.5 U (10.3 – 18.7) vs 20.0 U (17.3 – 26.8) vs 16 U (12 – 19) vs 12 U (8 – 22), p = 0.4). Event-free survival was significantly lower for group B (respective rates for Groups A, B, C, D were 87.5% vs 68.8% vs 96.3% vs 92.5%; p = 0.02). By multivariate Cox regression analysis, the CYP2C19 (+)*2/(−)*17 diplotype was an independent predictor of outcome, conferring a 5.2-fold higher adjusted risk for the composite endpoint than the others diplotypes. Conclusion: In our study, patients with the intermediate plus non-ultrarapid clopidogrel-metabolizing genotype ([+]*2/[−]*17) had a significantly poor medium-term prognosis for ischemic events, compared with the other diplotypes.

Keywords: CYP2C19*2/CYP2C19*17 Combination; Clopidogrel; Acute Coronary Syndrome; Prognosis

1. INTRODUCTION

Clopidogrel, a second-generation thienopyridine, is a pro-drug that requires enzymatic biotransformation into its active thiol metabolite before interacting with the P2Y12 receptor on platelets. Pharmacokinetic, pharmacodynamic, and genetic studies have provided abundant evidence that in vivo bioactivation of clopidogrel is a two-step process involving the cytochrome P450 (CYP) system. The isoenzyme CYP2C19 was found to play a major role, because it contributes to both clopidogrel bioactivation steps [1].

A common genetic variant within the CYP2C19 gene, the CYP2C19*2 loss-of-function polymorphism, was found to be associated with an attenuated response to clopidogrel [2-5], which resulted in worse clinical outcomes for patients undergoing coronary stenting [6,7] or with acute myocardial infarction (AMI) [8]. Poor prognosis was also demonstrated in a genome-wide association study [9] and in randomized clinical trials [10]. Although a 2012 meta-analysis questioned the relevance of the CYP2C19 loss-of-function alleles in the prediction of major cardiovascular events beyond stent thrombosis [11] a previous 2010 collaborative meta-analysis demon-
strated significant association between carriage of the
CYP2C19*2 allele and ischemic events [12].

The recently discovered allelic variant CYP2C19*17
results in increased CYP2C19 enzyme activity because of
the mutation (−806 C > T) in the 5′ flanking region of
the gene, which causes increased transcription of the en-
zyme [13]. Carriage of CYP2C19*17 was significantly
associated with enhanced response to clopidogrel and an
increased risk of bleeding in the context of percutaneous
coronary intervention [14], but was also found to confer
protection against ischemic events in patients after
AMI [15]. Moreover, it has also been reported that
combinations of the alleles CYP2C19*2 and CYP2C19*17
have significant effects on platelet aggregation [3], al-
though there have been no reports on prognosis regard-
ing ischemic events after an AMI. We therefore under-
took a study of patients carrying combinations of
CYP2C19*2 and CYP2C19*17 variant alleles, to determine medi-
unterm outcomes after an acute coronary syndrome
(ACS).

2. MATERIAL AND METHODS

2.1. Study Design and Eligibility

This was a prospective longitudinal selection of 220
consecutive patients admitted for an ACS between April
and October 2009, who survived hospitalization. Eligible
patients were younger than 75 years of age and were pre-
scribed clopidogrel (75 mg/day) and acetylsalicylic acid
(100 mg/day) at discharge.

Patients were excluded if they were immediately re-
ferred to surgery, or if they were enrolled in 2 other
clinical trials investigating antiplatelet therapy: The
TRA•CER (Trial to Assess the Effects of SCH 530348 in
Preventing Heart Attack and Stroke in Patients With
Acute Coronary Syndrome—ClinicalTrials.gov identifier:
NCT00527943) [16] and the TRILOGY ACS (A Com-
parison of Prasugrel and Clopidogrel in Acute Coronary
Syndrome Subjects—ClinicalTrials.gov identifier: NCT00699998) [17].

The initial study population included 220 patients.
In-hospital mortality was 3.6% (8 patients), and 78 pa-
tients were excluded because they were older than 75
years. During the study period, 24 patients were ran-
domized to TRA•CER and 5 to TRILOGY ACS. In ad-
dition, 10 patients were immediately referred to surgery.
The final study population included 95 patients. The in-
istitutional Ethics Committee approved the research pro-
tocol, and informed consent was obtained from the study
participants.

For analysis, the patients were divided into 4 groups
according to the CYP2C19*2 and CYP2C19*17 geno-
typed combination:

1) Group A ([+]2/[+]17): Intermediate plus ultra-
rapid metabolizers;
2) Group B ([+]2/[−]17): Intermediate plus non-ul-
trarapid metabolizers;
3) Group C ([−]2/[+]17): Non-intermediateplusul-
trarapid metabolizers;
4) Group D ([−]2/[−]17): Non-intermediate plus
non-ultrarapid metabolizers.

The phenotypic assignment presented was in accord-
dance to the clinical pharmacogenetics guideline for cy-
tochrome P450-2C19 genotype and clopidogrel therapy
[18].

Because there was a low number of homozygous pa-
tients, 1 patient with CYP2C19*2 and 3 patients with
CYP2C19*17, they were included in the heterozygous
Groups B and C, respectively, for analysis.

AMI was diagnosed based on the universal definition
of myocardial infarction as follows: 1) Positive for car-
diac biomarker (troponin I) with symptoms of ischemia
or 2) ECG changes indicating new ischemia (ST segment
and T wave changes and new bundle branch block) [19].

ST-elevation AMI was defined by a new onset ST eleva-
tion ≥0.2 mV for men and ≥0.15 mV for women in
V2-V3, and ≥0.1 mV in other leads. Non-ST elevation
AMI, in addition to the previously specified laboratory
and clinical criteria, may be associated with or without
ECG-associated ischemic changes, such as ST depres-
sion or T wave inversion [19].

Unstable angina was defined either by new-onset an-
gina (at least class III Canadian Cardiovascular Society
[CCS]); progressive angina; or angina at rest, with or
without ECG ischemic changes; plus a negative cardiac
biomarker assay [20].

The decision to allocate patients to an invasive or con-
servative management was performed by the cardiolo-
gists at the coronary care unit. A stress test was per-
formed in a third of the patients.

A 300 mg clopidogrel loading dose was administered
in the emergency department to patients with non-ST-
elevation ACS, and a 600 mg dose was administered to
patients with ST-elevation ACS. The subsequent daily
and discharge dose of clopidogrel was 75 mg adminis-
tered at 8 AM. Acetylsalicylic acid was administered at a
loading dose of 300 mg, and the subsequent daily dose
was 100 mg every day 4 hours after clopidogrel.

Platelet function testing was performed at discharge
using multiple electrode aggregometry (MEA) (Multi-
plate Analyzer®, Dynabyte, Munich, Germany). MEA
was assessed using whole blood, according to the princi-
ples of aggregometry; ADP (6.4 μmol/L) was used as the
agonist and results were expressed in arbitrary units (U).

2.2. Genotyping

Genomic DNA was extracted by standard methods at
hospital discharge from peripheral blood leukocytes. Patients were genotyped retrospectively. CYP2C19*1 (wild type) CYP2C19*2 (c.681G>A; rs4244285) and CYP2C19*3 (c.636G>A; rs4986893) alleles were identified using a dual-priming oligonucleotide polymerase chain reaction (PCR) assay from a commercially available kit (See gene®). A real-time PCR assay was used to identify the CYP2C19*17 allele (c.-806C>T; rs12248560), using a commercially available kit (Applied Bio systems®).

2.3. Baseline Data and Patient Follow Up

Admission data from patient records that included demographic, clinical, and laboratory information were analyzed. Information on medical therapy, catheterization, and discharge medication were also registered and analyzed.

Clinical follow up was performed over median of 136.0 (79.0 - 188.0) days after hospital discharge. Information was collected by phone calls to patients, from hospital records, or at the outpatient clinic. The primary endpoint used for analysis was a composite of cardiovascular death or readmission for ACS whichever came first. Clopidogrel compliance was confirmed during follow up.

2.4. Statistics

The Kolmogorov-Smirnov test was used to confirm that all continuous variables were normally distributed. Continuous data are presented as mean and standard deviation and groups were compared using analysis of variance (ANOVA) or the Student t test, when appropriate. Categorical variables are reported as frequencies and percentages, and the χ² or Fisher exact tests were used when appropriate. Chi square tables were used to compare the observed number of CYP2C19 genotypes with that expected for a population in Hardy-Weinberg equilibrium.

Cumulative survival curves were constructed using the Kaplan-Meier method, and patient groups were compared using the log-rank test. The period of observation started at hospital discharge.

A multivariate Cox regression analysis was performed for the primary endpoint. Variables that were significant at the bivariate level (with a p-value < 0.05) and that had clinical relevance were included in the model.

The study had a calculated post-hoc power of 46.3% to identify differences between the groups regarding the primary endpoint on the follow up.

All statistical tests were two-tailed, and a p-value less than 0.05 was considered significant. The analysis was performed using SPSS 15 (Statistical Package for Social Sciences) from SPSS Inc®, Chicago, IL. The post-hoc power calculation was performed with G*Power 3.1.

3. RESULTS

The mean age of the study patients was 59.9 ± 10.7 years with male gender predominance (83.2%). All were Caucasian. The frequencies of the CYP2C19*2 and CYP2C19*17 alleles were 14.2% and 20.0%, respectively. No deviations from the expected proportions of genotypes in the population as predicted by the Hardy-Weinberg equilibrium equation were noted (p = 0.44 for CYP2C19*2 and p = 0.61 for CYP2C19*17). The CYP2C19*3 variant alleles was not detected.

There were no significant differences between diploid groups regarding patient demographics, previous cardiovascular history, and risk factors. Group B had the lowest rate of non-ST elevation AMI as the admission diagnosis (respective rates for Groups A, B, C, D were 37.5% vs 11.1% vs 25.9% vs 50.0%; p = 0.02, Table 1).

### Table 1. Baseline characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>P*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61.3 ± 11.5</td>
<td>60.1 ± 10.3</td>
<td>60.6 ± 12.9</td>
<td>59.1 ± 9.3</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>7/8 (87.5)</td>
<td>14/18 (77.8)</td>
<td>24/27 (88.9)</td>
<td>34/42 (81.0)</td>
<td>0.74</td>
<td>0.50</td>
</tr>
<tr>
<td>STEMI (%)</td>
<td>4/8 (50.0)</td>
<td>7/18 (38.9)</td>
<td>14/27 (51.9)</td>
<td>11/42 (26.2)</td>
<td>0.16</td>
<td>0.92</td>
</tr>
<tr>
<td>NSTEMI (%)</td>
<td>3/8 (37.5)</td>
<td>2/18 (11.1)</td>
<td>7/27 (25.9)</td>
<td>21/42 (50.0)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>UA (%)</td>
<td>1/8 (12.5)</td>
<td>9/18 (50.0)</td>
<td>6/27 (22.2)</td>
<td>10/42 (23.8)</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>6/8 (75.0)</td>
<td>11/18 (61.1)</td>
<td>21/27 (77.8)</td>
<td>27/42 (64.3)</td>
<td>0.57</td>
<td>0.46</td>
</tr>
<tr>
<td>Dyslipidemia (%)</td>
<td>3/8 (37.5)</td>
<td>9/18 (50.0)</td>
<td>21/27 (77.8)</td>
<td>26/42 (61.9)</td>
<td>0.11</td>
<td>0.24</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>6/8 (75.0)</td>
<td>12/18 (66.7)</td>
<td>21/27 (77.8)</td>
<td>34/42 (81.0)</td>
<td>0.70</td>
<td>0.26</td>
</tr>
<tr>
<td>Current Smoking (%)</td>
<td>1/8 (12.5)</td>
<td>6/18 (33.3)</td>
<td>3/27 (11.1)</td>
<td>9/42 (21.4)</td>
<td>0.30</td>
<td>0.12</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>0/8 (0.0)</td>
<td>5/18 (27.8)</td>
<td>2/27 (7.4)</td>
<td>12/42 (28.6)</td>
<td>0.06</td>
<td>0.36</td>
</tr>
<tr>
<td>Previous PCI (%)</td>
<td>1/8 (12.5)</td>
<td>2/18 (11.1)</td>
<td>1/27 (3.7)</td>
<td>9/41 (22.0)</td>
<td>0.19</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*All groups comparison; **Group B vs rest population; Group A—[+]*2/[+]*17; Group B—[+]*2/[−]*17; Group C—[−]*2/[+]*17; Group D—[−]*2/[−]*17; STEMI—ST elevation acute myocardial infarction; NSTEMI—Non ST elevation acute myocardial infarction; UA—Unstable angina; MI—Myocardial infarction; PCI—Percutaneous coronary intervention.
Almost 75% of both sub-populations underwent invasive treatment. Patient groups had similar coronary anatomy, but Group B patients had the highest rate of stent placement (respective rates for Groups A, B, C, D were 42.9% vs 81.81% vs 81.0% vs 44.1%; p = 0.02). The groups were also similar with respect to ischemic and hemorrhagic risk assessments according to the Global Registry of Acute Coronary Events (GRACE), and the Can Rapid risk stratification of unstable angina patients Suppress adverse outcomes with early implementation of the ACC/AHA guidelines; LVEF—Left ventricular ejection fraction; CRP—C reactive protein; GFR—Glomerular filtration rate; Hg—Hemoglobin.

**Prognosis and Results of Multivariate Analysis**

Follow-up data were available for 91 patients (4.2% lost on follow-up). During follow up, there were 10 primary endpoints. One patient died, 6 had new nonfatal myocardial infarctions, and 3 were readmitted for unstable angina. Group B had the lowest event-free-survival rate (respective rates for Groups A, B, C, D were 87.5% vs 68.8% vs 96.3% vs 92.5%; log rank p < 0.02, [Figures 1-2]). After adjusting for covariates, the CYP2C19(*)2/*(-)17 diplotype was an independent predictor of outcome. It conferred a 5.2-fold higher adjusted risk for occurrence of the composite endpoint compared to the other diplotypes (Table 4).

### 4. DISCUSSION

The results of our study suggest that the diplotype combination CYP2C19*2 (intermediate clopidogrel metabolizer) and CYP2C19*17 (ultrarapid clopidogrel metabolizer) has prognostic significance over the medium term after an ACS. The patients with the diplotype CYP2C19 (+)*2/(-)*17 (intermediate and non-ultrarapid metabolizers) had higher risk for ischemic events following discharge compared to patients with the other diplotype s CYP2C19(+)*2/(+)*17 (intermediate and ultrarapid metabolizers), CYP2C19(-)*2/(-)*17 (non-intermediate and non-ultrarapid metabolizers), and CYP2C19(-)*2/(+)*17.

### Table 2. Risk assessment, management and laboratory data.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>P*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hospital stay (days)</strong></td>
<td>4.8 ± 2.3</td>
<td>4.8 ± 1.8</td>
<td>4.5 ± 1.4</td>
<td>4.4 ± 1.7</td>
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<tr>
<td><strong>Risk assessment</strong></td>
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<tr>
<td>GRACE score</td>
<td>127.0 ± 31.5</td>
<td>109.0 ± 26.8</td>
<td>113.9 ± 28.1</td>
<td>117.7 ± 22.4</td>
<td>0.52</td>
<td>0.28</td>
</tr>
<tr>
<td>Crusade score</td>
<td>20.6 ± 11.0</td>
<td>18.1 ± 14.6</td>
<td>20.0 ± 11.7</td>
<td>20.9 ± 12.3</td>
<td>0.89</td>
<td>0.47</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>62.4 ± 9.8</td>
<td>63.5 ± 14.1</td>
<td>62.1 ± 13.5</td>
<td>59.9 ± 15.8</td>
<td>0.88</td>
<td>0.57</td>
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<tr>
<td><strong>Cath lab data</strong></td>
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<tr>
<td>Invasive strategy (%)</td>
<td>7/8 (87.5)</td>
<td>11/18 (61.1)</td>
<td>21/27 (77.8)</td>
<td>34/42 (81.0)</td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td>One vessel disease (%)</td>
<td>4/7 (57.1)</td>
<td>6/11 (54.5)</td>
<td>9/21 (42.9)</td>
<td>13/34 (38.2)</td>
<td>0.69</td>
<td>0.44</td>
</tr>
<tr>
<td>Multivessel disease (%)</td>
<td>2/7 (28.6)</td>
<td>4/11 (36.4)</td>
<td>9/21 (42.9)</td>
<td>14/34 (41.2)</td>
<td>0.91</td>
<td>0.81</td>
</tr>
<tr>
<td>Stent (%)</td>
<td>3/7 (42.9)</td>
<td>9/11 (81.8)</td>
<td>17/21 (81.0)</td>
<td>15/34 (44.1)</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td><strong>Laboratory data</strong></td>
<td></td>
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</tr>
<tr>
<td>Peak Troponin I (U/l)</td>
<td>16.2 ± 22.5</td>
<td>10.2 ± 17.3</td>
<td>30.1 ± 41.0</td>
<td>21.2 ± 63.8</td>
<td>0.60</td>
<td>0.29</td>
</tr>
<tr>
<td>Peak CRP (mg/dl)</td>
<td>2.5 ± 2.3</td>
<td>2.9 ± 4.4</td>
<td>4.3 ± 5.3</td>
<td>4.2 ± 4.7</td>
<td>0.62</td>
<td>0.34</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>207.9 ± 60.1</td>
<td>196.4 ± 53.2</td>
<td>191.2 ± 46.9</td>
<td>189.6 ± 50.0</td>
<td>0.81</td>
<td>0.75</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>149.0 ± 45.8</td>
<td>133.7 ± 43.7</td>
<td>127.0 ± 37.3</td>
<td>128.6 ± 34.6</td>
<td>0.56</td>
<td>0.72</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>82.4 ± 30.4</td>
<td>98.2 ± 31.6</td>
<td>95.2 ± 40.0</td>
<td>100.0 ± 36.1</td>
<td>0.65</td>
<td>0.84</td>
</tr>
<tr>
<td>Hg variation (%)</td>
<td>9.2 ± 9.3</td>
<td>7.9 ± 11.7</td>
<td>5.9 ± 5.5</td>
<td>6.6 ± 6.2</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>Platelets variations (%)</td>
<td>17.7 ± 13.6</td>
<td>9.5 ± 11.8</td>
<td>10.8 ± 11.1</td>
<td>7.5 ± 8.5</td>
<td>0.70</td>
<td>0.91</td>
</tr>
<tr>
<td>ADP platelet aggregation (U)</td>
<td>14.2 ± 4.2***</td>
<td>19.3 ± 6.5</td>
<td>18.9 ± 6.5</td>
<td>18.9 ± 8.5</td>
<td>0.40</td>
<td>0.41</td>
</tr>
</tbody>
</table>

*All groups comparison; **Group B vs rest population; ***P value 0.03 for comparison with Group B; Group A—[+]*2/[+]*17; Group B—([-]*2/[+]*17); Group C—([-]*2/[-]*17); Group D—([-]*2/[+]*17); GRACE—Global registry of acute coronary events; CRUSADE—Can rapid risk stratification of unstable angina patients suppress adverse outcomes with early implementation of the ACC/AHA guidelines; LVEF—Left ventricular ejection fraction; CRP—C reactive protein; GFR—Glomerular filtration rate; Hg—Hemoglobin.
### Table 3. Medical therapy.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>P*</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At 24 hours</strong></td>
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<tr>
<td>Aspirin (%)</td>
<td>8/8 (100)</td>
<td>17/18 (94.4)</td>
<td>27/27 (100)</td>
<td>41/42 (97.6)</td>
<td>0.61</td>
<td>0.26</td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>8/8 (100)</td>
<td>18/18 (100)</td>
<td>27/27 (100)</td>
<td>41/42 (97.6)</td>
<td>0.74</td>
<td>0.63</td>
</tr>
<tr>
<td>LMWH (%)</td>
<td>7/8 (87.5)</td>
<td>14/18 (77.8)</td>
<td>21/27 (77.8)</td>
<td>28/42 (66.7)</td>
<td>0.22</td>
<td>0.74</td>
</tr>
<tr>
<td>GP IIb/IIIa inhibitors (%)</td>
<td>4/8 (50.0)</td>
<td>3/18 (16.7)</td>
<td>8/27 (29.6)</td>
<td>15/42 (35.7)</td>
<td>0.31</td>
<td>0.12</td>
</tr>
<tr>
<td>Beta blockers (%)</td>
<td>7/8 (87.5)</td>
<td>16/18 (88.9)</td>
<td>25/27 (92.6)</td>
<td>39/42 (92.8)</td>
<td>0.79</td>
<td>0.51</td>
</tr>
<tr>
<td>ACEi/ARBs (%)</td>
<td>8/8 (100)</td>
<td>18/18 (100)</td>
<td>26/27 (96.3)</td>
<td>39/42 (92.8)</td>
<td>0.74</td>
<td>0.39</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>8/8 (100)</td>
<td>18/18 (100)</td>
<td>27/27 (100)</td>
<td>42/42 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPI’s (%)</td>
<td>3/8 (37.5)</td>
<td>12/18 (66.7)</td>
<td>17/27 (63.0)</td>
<td>23/42 (54.8)</td>
<td>0.50</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>At discharge</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>8/8 (100)</td>
<td>18/18 (100)</td>
<td>27/27 (100)</td>
<td>42/42 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>8/8 (100)</td>
<td>18/18 (100)</td>
<td>27/27 (100)</td>
<td>42/42 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta blockers (%)</td>
<td>5/8 (62.5)</td>
<td>15/18 (83.3)</td>
<td>24/27 (88.9)</td>
<td>38/42 (90.5)</td>
<td>0.19</td>
<td>0.68</td>
</tr>
<tr>
<td>ACEi/ARBs (%)</td>
<td>7/8 (87.5)</td>
<td>18/18 (100)</td>
<td>24/27 (88.9)</td>
<td>39/42 (92.9)</td>
<td>0.51</td>
<td>0.18</td>
</tr>
<tr>
<td>Statins (%)</td>
<td>7/8 (87.5)</td>
<td>17/18 (94.4)</td>
<td>25/27 (92.6)</td>
<td>39/42 (92.9)</td>
<td>0.94</td>
<td>0.74</td>
</tr>
</tbody>
</table>

*All groupscomparison; **Group B vs rest population; Group A—([+]2/([+]2+[+]17]); Group B—([+]2/[-]+17]; Group C—([+]2/[-]+17]; Group D—([-]+2/[-]+17]; LMWH—Low molecular weight heparin; GP—Glycoprotein; ACEi—Angiotensin-converting-enzyme inhibitor; ARBs—Angiotensin II receptor blockers; PPIs—Proton pump inhibitors.

**Figure 1.** CYP2C19*2 and CYP2C19*17 diplotype combination and prognosis after an acute coronary syndrome.
Figure 2. Intermediate and non ultrarapid metabolizers and prognosis after an acute coronary syndrome.

Table 4. Predictors of the combined event.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>No event</th>
<th>Event</th>
<th>OR/Mean difference (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSTEMI†</td>
<td>28/81 (34.6)</td>
<td>2/10 (20.0)</td>
<td>2.11 (0.42 - 10.63)</td>
<td>0.36</td>
</tr>
<tr>
<td>Diabetes§</td>
<td>23/81 (28.4)</td>
<td>7/10 (70.0)</td>
<td>5.89 (1.40 - 24.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Previous myocardial infarction‡</td>
<td>17/81 (21.0)</td>
<td>2/9 (22.2)</td>
<td>1.08 (0.21 - 5.67)</td>
<td>0.91</td>
</tr>
<tr>
<td>CYP2C19 (–)§/2/–)§/17</td>
<td>37/81 (45.7)</td>
<td>3/10 (30.0)</td>
<td>0.51 (0.12 - 2.11)</td>
<td>0.35</td>
</tr>
<tr>
<td>CYP2C19 (+)§/2/–)§/17</td>
<td>11/81 (13.6)</td>
<td>5/10 (50.0)</td>
<td>6.34 (1.58 - 25.63)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CYP2C19 (–)§/2/+)§/17</td>
<td>33/81 (40.7)</td>
<td>2/10 (20.0)</td>
<td>0.36 (0.07 - 1.82)</td>
<td>0.20</td>
</tr>
<tr>
<td>CYP2C19 (+)§/2/+)§/17</td>
<td>7/81 (8.6)</td>
<td>1/10 (10.0)</td>
<td>1.18 (0.13 - 10.67)</td>
<td>0.87</td>
</tr>
<tr>
<td>Invasive strategy§</td>
<td>19/81 (23.5)</td>
<td>2/10 (20.0)</td>
<td>0.81 (0.16 - 4.17)</td>
<td>0.81</td>
</tr>
<tr>
<td>Multivessel disease‡</td>
<td>25/62 (40.3)</td>
<td>4/8 (50.0)</td>
<td>1.48 (0.34 - 6.48)</td>
<td>0.61</td>
</tr>
<tr>
<td>PPIs§</td>
<td>48/81 (59.3)</td>
<td>5/10 (50.0)</td>
<td>0.69 (0.18 - 2.56)</td>
<td>0.58</td>
</tr>
<tr>
<td>Age, years*</td>
<td>60.3 ± 10.9</td>
<td>60.2 ± 9.5</td>
<td>–0.1 (–7.3 - 7.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>60.4 ± 14.2</td>
<td>67.1 ± 15.3</td>
<td>6.8 (–3.5 - 17.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL*</td>
<td>3.5 ± 4.1</td>
<td>6.9 ± 7.7</td>
<td>3.5 (0.5 - 6.6)</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>GFR, mL/min/m²*</td>
<td>88.9 ± 35.8</td>
<td>79.5 ± 36.1</td>
<td>–10.5 (–34.4 - 13.4)</td>
<td>0.39</td>
</tr>
<tr>
<td>ADP platelet aggregation, U*</td>
<td>17.2 ± 7.8</td>
<td>23.4 ± 7.9</td>
<td>6.3 (1.1 - 11.5)</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

4.2 Multivariate cox regression analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>HR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 (+)§/2/–)§/17</td>
<td>5.17</td>
<td>1.35 - 19.83</td>
<td>0.02</td>
</tr>
<tr>
<td>Diabetes§</td>
<td>3.71</td>
<td>0.91 - 15.1</td>
<td>0.07</td>
</tr>
<tr>
<td>C-reactive protein (per 1 mg/dl increase)</td>
<td>1.12</td>
<td>0.91 - 1.5</td>
<td>0.23</td>
</tr>
<tr>
<td>ADP platelet aggregation (per 5 U increase)</td>
<td>1.84</td>
<td>0.99 - 3.41</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Chi square 21.6; p < 0.01

*mean ± SD; ‡%; PPIs—Proton pump inhibitors; LVEF—Left ventricular ejection fraction; NSTEMI—Non ST elevation acute myocardial infarction; GFR—Glomerular filtration rate.
(non-intermediate and ultrarapid metabolizers).

Using a candidate gene approach, Hulot et al were the first to report an association between the CYP2C19*2 genotype and ADP-stimulated platelet aggregation in response to clopidogrel [2]. Different genes have been implicated in clinically significant clopidogrel response; however, a recent genome-wide association study clarified the importance of the CYP2C19*2 genotype regarding the metabolism of clopidogrel [9]. A major locus on chromosome 10q24 that influences clopidogrel response extends across the CYP2C18-CYP2C19-CYP2C9-CYP2C8 gene cluster. Follow-up genotyping indicated that the common loss-of-function CYP2C19*2 variant was significantly associated with clopidogrel response and could account for most of the adverse events in the initial genome-wide association study. The CYP2C19*2 genotype accounted for approximately 12% of the variation in clopidogrel response and was associated with a worse ischemic outcome, while clinical variables such as age, body mass index, and lipid levels were responsible for approximately 10% of the variation in clopidogrel response [9]. An important conclusion from this analysis was that not only stent thrombosis, but also other events such as myocardial infarction, ischemic stroke, unplanned revascularization, and hospitalization for coronary ischemia without revascularization should be assessed with relation to the CYP2C19*2 genotype [21].

By contrast, carriage of the CYP2C19*17 allele was previously shown to be associated with increased activity of the CYP2C19 enzyme, leading to an increased rate of conversion of the pro-drug clopidogrel to an active compound, which was directly associated with a higher degree of platelet inhibition as analyzed by platelet aggregometry [14]. Assessment of a cohort of post-AMI patients who had undergone percutaneous intervention showed that carriage of CYP2C19*17 was associated with better clinical outcome regarding ischemic complications [15].

Previous authors have reported that the CYP2C19*2 and CYP2C19*17 variants, although 1995 base pairs apart are in linkage disequilibrium, and therefore are not independent of one another. Therefore, individuals heterozygous or homozygous for the *17 allele are less likely to carry the *2 allele, whereas those with no copies of the *17 allele (“wild type” at the *17 locus) are more likely to carry the *2 allele, supporting the concept that the *2 variant could account for most or all of the association with clopidogrel response at the CYP2C19 locus [22]. According to Gurbel et al., a hypothesis to overcome this disequilibrium would be to study the phenotypic and prognostic influence of CYP2C19*2/CYP2C19*17 diplotypes [22].

Sibbing et al. first reported on the phenotypic association of clopidogrel-induced platelet aggregation with the diplotype CYP2C19*2 plus CYP2C19*17. Using platelet aggregometry in patients chronically medicated with clopidogrel, the authors reported that a gradual increase in platelet aggregation was seen, starting with the (-)*2/ (+)*17 patients who had the lowest ADP-induced platelet aggregation, to the (-)*2/(-)*17 and (+)*2/(+) patients, and ending with the (+)*2/(-)*17 patients, who exhibited the highest value. It was concluded that the increased platelet aggregation seen with CYP2C19*2 was in part reduced by the concomitant presence of CYP2C19*17, although no ischemic or bleeding events were reported for this gene interaction among the 986 stable post-PCI patients with coronary artery disease [3]. A recent paper by Harmsze et al confirmed the phenotypic changes seen for this diplotype. They measured platelet aggregation using the Verify Now® and the light transmission aggregation assays, and the results were consistent with the hypothesis that the effect of CYP2C19*2 effect was partly reduced by the concomitant presence of the CYP2C19*17 allele. However, their in vitro findings were not corroborated with decreased risk of bleeding events or increased ischemic events. The authors proposed that CYP2C19*2 leads to a complete loss of enzyme function, whereas CYP2C19*17 only enhances existing enzyme capacity [23].

None of the studies we have discussed included findings on the risk of ischemic events in patients with the diplotype CYP2C19*2 plus CYP2C19*17. Although the association between this diplotype and platelet aggregation has been previously reported, in our study, the results of platelet aggregation were not statistically significant for all groups comparison because of the low statistical power of our study with respect to platelet aggregation. Nevertheless the (+)*2/(+) patients had a significant higher platelet reactivity than the (-)*2/(-) patients.

Our study patients represent a real-world ACS cohort, with low risk for bleeding, and an intermediate-to-high risk for ischemic events, probably related to the exclusion of patients older than 75 years of age. Although previous studies have identified significant differences between the allelic and genotypic frequencies of polymorphisms (CYP2C19*2 and CYP2C19*17) associated to clopidogrel according to ethnicity [24], our patient population only included Caucasians. We note that the patients with the intermediate- plus- ultrarapid clopidogrel-metabolizing genotype (Group B) had the highest rate of percutaneous coronary intervention for stent placement. Therefore increased platelet reactivity may have been critical for that group of patients, influencing their adverse outcomes with regard to ischemic events. Our data indirectly supports the concept that the loss of function CYP2C19*2 allele is partly compensated by the concomitant presence of CYP2C19*17, resulting in
clinically significant outcomes after ACS.

5. CONCLUSION
The CYP2C19*2 plus CYP2C19*17 diplotype combination had a significant medium-term effect on the occurrence of ischemic events in patients taking clopidogrel following an acute coronary syndrome.

6. ACKNOWLEDGEMENTS
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REFERENCES


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