Genotyping of six clopidogrel-metabolizing enzyme polymorphisms has a minor role in the assessment of platelet reactivity in patients with acute coronary syndrome

María Henar García-Lagunar, Luciano Consuegra-Sánchez*, Pablo Conesa-Zamora**, Javier Ruiz-Cosano***, Federico Soria Arcos*, Luis García de Guadiana**, Pedro Cano Vivar*, Juan Antonio Castillo-Moreno*, Antonio Melgarejo-Moreno*

Departments of Hospital Pharmacy, *Cardiology, **Clinical Analysis, ***Pathology
Santa Lucía General University Hospital (HGUSL); Cartagena-Spain

Introduction

Although the current guidelines for acute coronary syndrome (ACS) give preference to ticagrelor and prasugrel, a lot of ACS patients continue to receive clopidogrel as medical treatment (1). However, despite dual antiplatelet therapy, a large number of patients present incomplete platelet inhibition (2, 3), and a high residual platelet reactivity on clopidogrel, also termed poor response to clopidogrel (PRC), is associated with increased cardiovascular ischemic events and an unfavorable prognosis (4). Mechanisms contributing to PRC are not entirely well known and are probably multifactorial (5–7).

Clopidogrel is a prodrug that requires biotransformation to generate an active metabolite. It is metabolized by the hepatic cytochrome P450 (CYP1A2, CYP2B6, and CYP2C19) and transformed into the intermediate metabolite, 2-oxo-clopidogrel, which is further oxidized by various isoenzymes (CYP2B6, CYP2C9, CYP2C19, and CYP3A4) and paraoxonase 1 (PON1) into an inactive carboxyl group and a highly unstable active thiol derivative (Fig. 1) (8).

To date, there have been few studies (9–15) that evaluate a potential association between CYP3A4*1B allele and response to clopidogrel. These studies (9–12, 14, 15) were conducted in healthy subjects (15), in patients with stable coronary artery disease (10), those undergoing elective percutaneous coronary intervention (9, 12), patients with a history of stent thrombosis (11), or mixed patient populations with stable and unstable coronary artery disease (14). Only one previous study (13) has been
carried out in patients with ACS, and this is a special population with clinical and inflammatory peculiarities (16); although this study did not find a relationship between CYP3A4*1B and platelet reactivity, the statistical adjustment for clinical variables was relatively incomplete.

The most studied polymorphisms related to clopidogrel metabolism are found in CYP2C9, CYP2C19, and PON1 genes (2, 17, 18). However, results of their influence on platelet reactivity have been contradictory, with CYP2C19*2 being often associated with PRC (2, 18, 19). Incomplete adjustment by confounders partly accounts for the different findings. For example, in a recent study (20) evaluating 25 polymorphisms, only a limited set of potential confounders [i.e., age, gender, cardiovascular risk factors, body mass index (BMI) and proton-pump inhibitors] was analyzed. However, it was concluded that CYP2C19*2 allele tagged-SNP (single-nucleotide polymorphism) rs4244285 was a “strong” predictor of PRC. Further, no incremental value on prediction of PRC (above clinical variables) was provided by the authors. In this regard, a consensus is needed for statistical methods to properly assess the incremental value of a number of SNPs single polymorphisms or a genetic risk score in clinical practice (21). One set of metrics proposed for the assessment of novel markers in general, but not specifically for genetic markers, includes discrimination capacity (22). However, to date, only a limited number of prospective studies have assessed the incremental benefits (i.e., discrimination) of the genetic risk score over and abovementioned known clinical risk predictors (23).

Thus, in this study, we evaluated the contribution of clopidogrel-metabolizing enzyme polymorphisms on platelet reactivity in patients with ACS treated with clopidogrel over and above clinical and laboratory variables.

Methods

Population

We conducted an observational study, with cross-sectional analysis and prospective/consecutive data collection between June 2011 and January 2012. We included patients diagnosed with ACS, defined as typical chest pain and elevated markers of myocardial necrosis or T/ST-segment alterations suggestive of ischemia, remitted for cardiac catheterization and treated with clopidogrel ≥12 h, with a loading dose of 300 or 600 mg (physician choice).

In cases where clopidogrel loading dose could not be confirmed, patients were included if they were treated for at least 24 h after the first. Collected data for each patient encompassed baseline characteristics, including comorbidities and concomitant treatment.

Exclusion criteria were the presence of significant valvular heart disease or cardiomyopathy, concomitant diseases with life expectancy of <1 year, patients who did not sign the informed consent, and patients treated with platelet glycoprotein IIb/IIIa receptor antagonists. The study was approved by the Ethics Committee for Clinical Research at our center, and it complies with the Helsinki Declaration of 1975 and subsequent updates.

Platelet function

At the hemodynamic laboratory, we extracted 15 mL of peripheral blood from arterial sheath before using anticoagulants. We filled two tubes containing 3.2% sodium citrate (Vacuette®) and waited between 15 and 30 min before the evaluations, according to the manufacturer’s instructions. The inhibitory effect of clopidogrel on platelet reactivity was measured with VerifyNow P2Y12® (Accumetrics Inc. San Diego, CA, USA). The instrument measures the change in light transmittance and the results were expressed as “Base PRU (Platelet Reactivity Units)”: an estimate of the patient’s baseline platelet function independent of P2Y12 receptor inhibition, “PRU”: the amount of P2Y12 receptor-mediated aggregation, and “Percent inhibition [(PRU−Base PRU)/Base PRU × 100]”: the difference between before and after clopidogrel treatment platelet reactivity. We used the cut-off level PRU=208 specified by the manufacturer as the definition of poor responders (24).

Genotyping

Peripheral blood samples were obtained from arterial sheath in EDTA tubes and DNA was extracted using the QIAamp® DNA minikit and automatic nucleic acid extractor QiaCube® (Qiagen, Hilden, Germany). Six SNPs tagging alleles involved in the me-

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Figure 1. Hepatic metabolism of clopidogrel showing the enzymes involved. Those indicated with thick edges are coded by genes whose polymorphisms have been studied in this work. With permission by PharmGKB, the Pharmacogenomics Knowledgebase (25)
Genotyping and clopidogrel metabolism of clopidogrel were studied: CYP2C9*2 (rs1799853), CYP2C9*3 (rs1057910), CYP2C19*2 (rs4244285), CYP2C19*17 (rs12248560), CYP3A4*1B (rs27405749), and PON1-Q192R (rs662) (25). Genotyping were determined by allelic discrimination using the TaqMan® Drug Metabolizing and the reactive GTXpress Master Mix (CYP2C19*2, CYP2C19*17 and PON1-Q192R), provided by Applied Biosystems (Foster City, CA, USA), or KASP® (CYP2C9*2, CYP2C9*3 and CYP3A4*1B) based on FRET technology (Kbiosciences, Hertfordshire, UK).

Statistical analysis
Univariate analysis was performed using the chi-square test or Fisher’s test for categorical variables and the Student’s t-test for continuous variables to identify factors associated with PRC. PRC (PRU >208) was the dependent variable in the models of binary logistic regression. Covariates were those that showed association in the univariate analysis (p<0.05) or in previous studies under an explanatory perspective (age, gender, BMI, current smoking, type-2 diabetes mellitus, heart failure, acute myocardial infarction, estimated glomerular filtration rate, hemoglobin levels, concomitant statins and calcium-channel blockers). Covariates were entered in blocks using the backward stepwise method, applying the Wald statistic. Polymorphisms were introduced in a second block using the “enter” option. Odds ratios (OR) were calculated with their respective 95% confidence intervals (95% CI). The discrimination of the final model with and without the genetic score (six polymorphisms) was estimated using the C statistic, and the calibration using the Hosmer–Lemeshow test. Chi-squared score of each variable was estimated in the model as a method to assess the relative importance of each variable in the model. The first-degree interaction in the hierarchical model between variables loading dose and polymorphisms independently associated with PRC was analyzed. In addition, the C statistic for the model that only included clinical variables was compared by a hypothesis contrast test. A p value of <0.05 was considered statistically significant and all analyses were performed using the SPSS statistical package, version 20.0 (IBM, USA).

Results
Study population
We included 278 patients with a mean age of 66 years (standard deviation 11 years), and 85 (30.6%) were women. The baseline characteristics of the study sample are shown in Table 1. The diagnosis was ACS without persistent ST-segment elevation in 182 patients (65.5%), whereas it was ACS with persistent ST-segment elevation in 56 (20.1%). Poor responders had a significantly higher age; had a higher incidence of heart failure (Killip class >I); and had lower hematocrit, hemoglobin, and estimated glomerular filtration values were lower.
Troponin I elevation above the laboratory reference value was observed in 177 cases (63.7%). In 159 patients (57.2%), clopidogrel loading dose was administered, with 300 mg being the most common dose (n=139, 87.4%). The mean time from the first dose of clopidogrel until the determination of platelet aggregation was almost 9 days and the median was 5 days (interquartile range 2–10). At study enrolment, 231 (83.1%) were clopidogrel naïve patients (Table 2).
Concomitant medications are also listed in Table 2. Of note, in 105 cases (37.8%), the patient received a proton-pump inhibitor, with pantoprazole being the most common (n=67, 63.8%); the most frequently used dose was 40 mg every 24 h (n=42, 62.7%). In 231 patients (83.1%), statins were administered, with a predominance of atorvastatin (n=215, 93.1%) 80 mg every 24 h (n=117, 54.4%). There were no significant differences in the use of concomitant medications during hospitalization between patients with and without PRC.

The angiographic characteristics are presented in Table 2. The mean number of vessels with significant lesions was 1.5±1 and the mean number of coronary lesions treated was 1.1±0.9 with 1.2±1.0 stents per person.

Patient profile with high on-treatment platelet reactivity
Overall PRU mean value was 261±78. According to response to clopidogrel, PRU was 164±35 in patients with adequate response and 295±58 in poor responders. We identified 206 (74.1%) poor responders. In univariate analysis with platelet response (PRU>208 U) as the dichotomizing variable, we found that age (OR 1.63 per standard deviation, 95% CI 1.24–2.15), heart failure (OR 4.83, 95% CI 1.12–20.95), and the presence of ≥1 CYP2C19*2 allele (OR 2.01, 95% CI 1.03–3.93) were risk factors for high platelet reactivity. Current smoking (OR 0.41, 95% CI 0.23–0.72), hemoglobin (OR 0.65, 95% CI 0.54–0.77), hematocrit (OR 0.86, 95% CI 0.80–0.92), and estimated glomerular filtration rate (mL/min/1.73 m², evaluated with the Modification of Diet in Renal Disease formula OR 0.99, 95% CI 0.98–1.00) were found as protective factors. In a multiple logistic regression model that included age, sex, BMI, diabetes mellitus, current smoking, heart failure, acute myocardial infarction, baseline hemoglobin, estimated glomerular filtration rate, concomitant medication (calcium-channel blockers and statins), and the six polymorphisms (Table 3), the following were independent predictors of PRC risk: age (OR 1.43 per standard deviation, 95% CI 1.03–2.00), BMI (OR 4.03 per standard deviation, 95% CI 1.21–13.43), and the presence of ≥1 CYP3A4*1B allele (OR 4.05, 95% CI 1.01–16.34); on the other hand, high baseline hemoglobin (OR 1xe–18, 95% CI 1xe–26–1xe–10) was a protective factor. Female gender showed a tendency for being a protective factor (OR 0.44, 95% CI 0.20–1). The p value of Hosmer–Lemeshow test was 0.833. Chi-square score evaluation reported that (in order of importance) the most important variable in the model was hemoglobin, followed by age, CYP2C9*2, CYP2C9*2, and CYP3A4*1B.

Polymorphisms. Frequency and impact on platelet reactivity
The frequencies of CYP3A4*1B, CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*17, and PON1-Q192R polymorphisms are
shown in Table 4. Polymorphisms were in Hardy–Weinberg equilibrium (p=0.798, p=0.566, p=0.388, p=0.925, p=0.469, and p=0.210, respectively). The most common polymorphism in our study was the presence of at least one C allele of PON1- Q192R (55.0), whereas the most uncommon was the presence of at least one *1B allele of CYP3A4 (8.3%). The other polymorphisms showed frequencies between 16.2% and 38.1%. Poor responders presented a higher prevalence of CYP2C19 polymorphism and a tendency to significance was observed for CYP2C9*2 polymorphism among normal responders. There were not differences between patients with and without PRC for the rest of polymorphisms.

Table 5 shows the aggregometry results associated with the six polymorphisms. The patients with at least one CYP2C19 allele had significantly higher PRU values than patients with the original genotype, and the percent inhibition

### Table 1. Baseline characteristics

|                          | Total cohort (n=278) | Poor responders (n=206, 74.1%) | Normal responders (n=72, 25.9%) | P       |
|--------------------------|---------------------|---------------------------------|---------------------------------|---------|
| Age, years               | 65.9±11.2           | 67.3±11.0                       | 61.9±11.0                       | <0.001  |
| Female gender            | 85 (30.6)           | 66 (32.0)                       | 19 (26.4)                       | 0.370   |
| BMI, kg/m²               | 30.0±15.8           | 30.6±18.2                       | 28.2±4.2                        | 0.265   |
| **Risk factors**         |                     |                                 |                                 |         |
| Arterial hypertension    | 179 (64.4)          | 137 (66.5)                      | 42 (58.3)                       | 0.213   |
| Dyslipidemia             | 162 (58.3)          | 121 (58.7)                      | 41 (56.9)                       | 0.791   |
| Diabetes mellitus        | 108 (38.9)          | 83 (40.3)                       | 25 (34.7)                       | 0.404   |
| Current smoking          | 86 (30.9)           | 53 (25.7)                       | 33 (45.8)                       | 0.001   |
| **Comorbidities**        |                     |                                 |                                 |         |
| FH ischemic heart disease| 23 (8.3)            | 14 (6.8)                        | 9 (12.5)                        | 0.130   |
| Ischemic heart disease   | 121 (43.5)          | 91 (44.2)                       | 30 (41.7)                       | 0.712   |
| PPCA                     | 84 (30.2)           | 61 (29.6)                       | 23 (31.9)                       | 0.711   |
| Aortoconary bypass       | 12 (4.3)            | 9 (4.4)                         | 3 (4.2)                         | 1       |
| Stroke                   | 10 (3.6)            | 8 (3.9)                         | 2 (2.8)                         | 1       |
| Peripheral artery disease| 11 (4.0)            | 7 (3.4)                         | 4 (5.6)                         | 0.483   |
| COPD                     | 18 (6.5)            | 16 (7.8)                        | 2 (2.8)                         | 0.172   |
| CKD                      | 19 (6.8)            | 17 (8.3)                        | 2 (2.8)                         | 0.173   |
| **Hospitalization**      |                     |                                 |                                 |         |
| Killip class > I         | 27 (9.7)            | 25 (12.1)                       | 2 (2.8)                         | 0.021   |
| Changes in the ECG*      | 174 (62.6)          | 131 (63.6)                      | 43 (59.7)                       | 0.559   |
| Troponin I elevation     | 177 (63.7)          | 126 (61.2)                      | 51 (70.8)                       | 0.142   |
| **Laboratory data**      |                     |                                 |                                 |         |
| Hemoglobin, g/dL         | 13.8±1.9            | 13.4±1.9                        | 14.7±1.7                        | <0.001  |
| Hematocrit, %            | 41.2±5.0            | 40.4±4.9                        | 43.7±4.6                        | <0.001  |
| Platelets, μ10⁹/L        | 212.1±55.0          | 214.9±56.0                      | 204.1±51.7                      | 0.153   |
| Leukocytes, ×10⁹/L       | 9.2±8.4             | 9.6±9.5                         | 8.2±2.9                         | 0.228   |
| Creatinine, mg/dL        | 1.0±0.5             | 1.0±0.5                         | 0.9±0.3                         | 0.092   |
| MDRD, mL/min/1.73 m²     | 84.0±28.8           | 81.4±26.5                       | 91.3±33.7                       | 0.013   |
| Total cholesterol, mg/dL | 176.0±48.5          | 172.1±47.0                      | 186.2±51.1                      | 0.045   |
| HDL cholesterol, mg/dL   | 38.3±12.0           | 37.7±11.8                       | 39.7±12.7                       | 0.263   |
| LDL cholesterol, mg/dL   | 107.3±39.2          | 105.6±39.2                      | 111.8±39.1                      | 0.294   |
| Triglycerides, mg/dL     | 150.5±94.2          | 142.6±70.9                      | 171.4±11.0                      | 0.055   |

Quantitative variables are presented as mean±standard deviation and categorical variables as frequency and percentage. BMI - body mass index; CKD - chronic kidney disease; C0DP - chronic obstructive pulmonary disease; ECG - electrocardiogram; FH - ischemic heart disease-Family history of ischemic heart disease; HDL - high-density lipoprotein; Killip class - presence of heart failure according to Killip and Kimball classification; LDL - low-density lipoproteins; MDRD - glomerular filtration rate according to the formula Modification of Diet in Renal Disease Brief; PPCA - Previous Percutaneous Coronary Angioplasty. *J point deviation ≥1 mm and/or presence of negative T-wave symmetry ≥3 mm except avR.
| Concomitant treatment during hospitalization | Total cohort (n=278) | Poor responders (n=206, 74.1%) | Normal responders (n=72, 25.9%) | P |
|--------------------------------------------|----------------------|--------------------------------|-----------------------------|----|
| **Salicylates**                            | 278 (100)            | 206 (100)                      | 72 (100)                    | 1  |
| **H2RAs**                                  | 102 (36.7)           | 75 (36.4)                      | 27 (37.5)                   | 0.890 |
| **PPIs**                                   | 105 (37.8)           | 80 (38.8)                      | 25 (34.7)                   | 0.517 |
| **ACE inhibitors or ARBs**                 | 206 (74.1)           | 149 (71.6)                     | 57 (79.2)                   | 0.225 |
| **Loop diuretics**                         | 51 (18.4)            | 43 (20.9)                      | 8 (11.1)                    | 0.061 |
| **Alpha-blockers**                         | 9 (3.2)              | 6 (2.9)                        | 3 (4.2)                     | 0.701 |
| **Beta-blockers**                          | 210 (75.5)           | 153 (74.3)                     | 57 (79.2)                   | 0.476 |
| **Nitrates**                               | 95 (34.2)            | 71 (34.5)                      | 24 (33.3)                   | 0.801 |
| **CCBs**                                   | 45 (15.1)            | 36 (17.5)                      | 9 (12.5)                    | 0.295 |
| **Statins**                                | 231 (83.1)           | 168 (81.6)                     | 63 (87.5)                   | 0.309 |
| **Aldosterone antagonist**                 | 14 (5.0)             | 12 (5.8)                       | 2 (2.8)                     | 0.532 |

| **Clopidogrel**                            |                      |                                |                            |    |
| **Clopidogrel-naïve patients**             | 231 (83.1%)          | 174 (84.5)                     | 57 (79.2)                   | 0.497 |
| **Time from the first dose, days**         | 8.5±14.71            | 8.75±16.28                     | 9.14±8.9                    | 0.846 |
| **Loading dose**                           | 159 (57.2)           | 116 (56.3)                     | 43 (59.7)                   | 0.621 |
| **Loading dose of 300 mg**                 | 139 (87.4)           | 100 (86.2)                     | 39 (90.7)                   | 0.760 |

| **Cardiac catheterization**                |                      |                                |                            |    |
| **Depressed LVEF**                         | 55 (19.8)            | 46 (22.3)                      | 9 (12.5)                    | 0.076 |
| **Number of diseased vessels**             | 1.5±1.1              | 1.4±1.1                        | 1.4±1.0                     | 0.345 |
| **LMCA**                                   | 16 (5.8)             | 12 (5.8)                       | 4 (5.6)                     | 1    |
| **LAD or its branches**                    | 153 (55.0)           | 115 (55.8)                     | 38 (52.8)                   | 0.823 |
| **Cx or its branches**                     | 106 (38.1)           | 76 (36.9)                      | 30 (41.7)                   | 0.375 |
| **RCA or its branches**                    | 137 (49.3)           | 109 (52.9)                     | 28 (38.9)                   | 0.662 |
| **Grafts**                                 | 7 (2.5)              | 6 (2.9)                        | 1 (1.4)                     | 0.683 |
| **Treated lesions**                        | 1.1±0.9              | 1.1±0.8                        | 1.2±1.1                     | 0.594 |
| **Stents, units**                          | 1.2±1.0              | 1.2±1.0                        | 1.3±1.3                     | 0.442 |
| **≥1 Pharmacoactive stent**                | 149 (53.6)           | 110 (53.4)                     | 39 (54.2)                   | 0.583 |
| **Total length, mm**                       | 29.2±18.2            | 29.0±17.2                      | 29.9±21.1                   | 0.765 |

| **Treatment at discharge**                 |                      |                                |                            |    |
| **Salicylates**                            | 250 (89.9)           | 184 (89.3)                     | 66 (91.7)                   | 0.461 |
| **Clopidogrel**                            | 213 (76.6)           | 156 (75.7)                     | 57 (79.2)                   | 0.437 |
| **Prasugrel**                              | 11 (4.0)             | 11 (5.3)                       | 0 (0)                       | 0.072 |
| **H2RAs**                                  | 48 (17.3)            | 38 (18.5)                      | 10 (13.9)                   | 0.313 |
| **PPIs**                                   | 100 (36.0)           | 75 (36.4)                      | 25 (34.7)                   | 0.637 |
| **ACE inhibitors or ARBs**                 | 207 (74.5)           | 152 (73.8)                     | 55 (76.4)                   | 0.946 |
| **Loop diuretics**                         | 48 (17.3)            | 41 (19.9)                      | 7 (9.7)                     | 0.040 |
| **Alpha-blockers**                         | 8 (2.9)              | 6 (2.9)                        | 2 (2.8)                     | 1    |
| **Beta-blockers**                          | 212 (76.3)           | 154 (74.8)                     | 58 (80.6)                   | 0.473 |
| **Nitrates**                               | 41 (14.8)            | 30 (14.6)                      | 11 (15.3)                   | 0.956 |
| **CCBs**                                   | 44 (15.8)            | 35 (17.0)                      | 9 (12.5)                    | 0.319 |
| **Statins**                                | 231 (83.1)           | 171 (83.0)                     | 60 (83.3)                   | 0.553 |
| **Aldosterone antagonist**                 | 11 (4.0)             | 10 (4.9)                       | 1 (1.4)                     | 0.298 |
| **Acenocumarol**                           | 12 (4.3)             | 10 (4.9)                       | 2 (2.8)                     | 0.738 |

Quantitative variables are presented as mean±standard deviation and categorical variables as frequency and percentage. ACE - inhibitors-angiotensin-converting-enzyme inhibitors; ARBs - angiotensin II receptor blockers; CCBs - calcium-channel blockers; Cx - circumflex artery; H2RAs - Histamine-2 receptor antagonists; LAD - left anterior descending artery; LMCA - left main coronary artery; LVEF - left ventricular ejection fraction; PPIs - proton-pump inhibitors; RCA - right coronary artery

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Table 3. Clinical-genetic model for prediction poor response to clopidogrel

| Clinical variables                           | Adjusted OR | 95% CI        | P   | Chi² |
|---------------------------------------------|-------------|---------------|-----|-----|
| Age, per each SD                            | 1.43        | 1.03–2.00     | 0.034 | 11.28 |
| Female gender                               | 0.44        | 0.20–1.00     | 0.050 | 0.31 |
| BMI, per each SD                             | 4.03        | 1.21–13.43    | 0.024 | 1.43 |
| Hemoglobin, g/dL                           | 1×10⁻¹⁸     | 1×10⁻⁸–1×10⁻¹⁰ | <0.001 | 23.17 |
| Concomitant statins                          | 0.43        | 0.17–1.09     | 0.074 | 0.64 |

**Genetic variables**

- CYP3A4 ≥1 allele 1B: 4.05 (1.01–16.34, 0.049, 1.72)
- CYP2C9 ≥1 allele *2: 0.62 (0.32–1.23, 0.170, 3.94)
- CYP2C9 ≥1 allele *3: 1.35 (0.55–3.35, 0.517, 0.26)
- CYP2C19 ≥1 allele *17: 1.73 (0.83–3.59, 0.145, 1.61)
- CYP2C19 ≥1 allele *2: 2.03 (0.92–4.50, 0.081, 2.77)
- PON1 Q192R ≥1 allele C: 0.54 (0.18–1.67, 0.287, 1.54)

*Variable transformed by the logarithm of decimal base; *91.3% were atorvastatin takers; *Adjusted for current smoking, diabetes, heart failure, acute myocardial infarction, estimated glomerular filtration rate and concomitant calcium-channel blockers. BMI - body mass index; CI - confidence interval; OR - odds ratio; SD - standard deviation.

Model calibration: Hosmer–Lemeshow test: χ²=4.256; df=8; P value=0.833

Table 4. Proportion of polymorphisms related to hepatic metabolism of clopidogrel

| Polymorphism                                      | Total cohort (n=278) | Poor responders (n=206, 74.1%) | Normal responders (n=72, 25.9%) | P     |
|---------------------------------------------------|---------------------|--------------------------------|---------------------------------|-------|
| CYP3A4 ≥1 allele 1B, n (%)                        | 23 (8.3)            | 19 (9.2)                       | 4 (5.6)                         | 0.354 |
| CYP2C9 ≥1 allele *2, n (%)                        | 90 (32.4)           | 61 (29.6)                      | 29 (40.3)                       | 0.071 |
| CYP2C9 ≥1 allele *3, n (%)                        | 45 (16.2)           | 35 (17.0)                      | 10 (13.9)                       | 0.596 |
| CYP2C19 ≥1 allele *17, n (%)                      | 106 (38.1)          | 83 (40.3)                      | 23 (31.9)                       | 0.209 |
| CYP2C19 ≥1 allele *2, n (%)                       | 76 (27.3)           | 63 (30.6)                      | 13 (18.1)                       | 0.038 |
| PON1-Q192R ≥1 allele C, n (%)                     | 153 (55.0)          | 111 (53.9)                     | 42 (58.3)                       | 0.539 |

Qualitative variables are presented as frequency and percentage.

Discussion

The main result of our study suggests that the presence of at least one CYP3A4*1B allele can independently influence platelet response to clopidogrel in patients with ACS in an exhaustively adjusted model. The relative importance of CYP3A4*1B as a predictor of response to clopidogrel is modest compared with clinical or routine laboratory variables such as age, sex, BMI, and baseline hemoglobin, as suggested the analysis of discrimination and the chi-square score. Importantly, our analysis further shows that a clinical model built with five (age, gender, BMI, hemoglobin, and concomitant statins) easily obtained variables yielded a good discrimination in the identification of patients with poor response to clopidogrel, and even though the discrimination significantly improves when a set of six polymorphisms is added compared to the clinical model, this increase is poor.

There have been few studies that evaluate the effect of CYP3A4*1B on the response to clopidogrel (9–15). Of these, only one previous study (13) has evaluated the prognostic value of CYP3A4*1B in patients with ACS under similar conditions to ours. That study recruited 603 patients with ACS without ST-segment elevation and yielded a negative result (13); remarkably, the analysis for prediction of platelet reactivity was performed by adjusting it only for age and sex. Nevertheless, in our study, the model was carefully adjusted for age, sex, BMI, diabetes, current smoking, heart failure, acute myocardial infarction, baseline hemoglobin, estimated glomerular filtration rate, concomitant medication, and the six polymorphisms. These substantial differences in the statistical adjustment may have had a role in the discordance between the findings of the two studies. The remaining studies have been conducted in other patient types (9–12, 14, 15).

Some studies have shown that other CYP3A4 polymorphisms influence the response to clopidogrel (2, 10). However, the associations for polymorphism CYP3A4*1B could not be confirmed (9–12, 14, 15); this may be because of the small sample size of the studies (9, 10, 15) and the low prevalence of the polymorphism (9, 10), which did not allow for the analysis.

Moreover, Brandt et al. (15) performed a study in healthy subjects and Angelillo et al. (10) in patients with stable coronary artery disease. Our results are not comparable to those found among wild-type carriers.

Discrimination of poor response to clopidogrel: clinical and genetic variables

The C statistic for the model presented above was 0.749 (95% CI 0.683–0.815) and for the model that included CYP3A4*1B was 0.763 (95% CI 0.699–0.826, p for area comparison=0.088).

Furthermore, the C statistic for the model that included clinical variables and the six polymorphisms was 0.788 (95% CI 0.729–0.847, p for area comparison versus clinical model=0.028) (Fig. 2). The increment of the discrimination capacity compared to the clinical model was 3.9%.
in patients undergoing “elective” coronary intervention (9, 12), those by Geisler et al. (14) found in a mixed population in which only 45.5% had ACS, or those of Harmsze et al. (11) found in study with a different design—cases and controls—in patients with a history of stent thrombosis. Our study, on the other hand, was conducted in a population of ACS patients referred for cardiac catheterization.

Several studies have associated the CYP2C19*2 polymorphism with higher values of PRU and an increase of cardiovascular adverse effects (17, 18, 26). However, in our analysis, it was not an independent predictor or PRC, although it presented the second highest chi-square score of genetic variables. Our results are in contrast with those of Park et al. (2), which examined the relationship between a panel of clinical variables and nine

| Table 5. Aggregometry results for polymorphisms related to hepatic metabolism of clopidogrel and platelet reactivity |
|--------------------------------------------------|
| CYP3A4                                           |
| wt/wt (n=252, 90.7%)                              |
| wt/*1B (n=23, 8.3%)                               |
| *1B/*1B (n=0)                                    |
| P                                                |
| Base PRU                                         |
| 300.0±54.5                                       |
| PRU                                              |
| 284.6±64.8                                       |
| 261.4±78.9                                       |
| PRU <208                                         |
| 267.0±67.8                                       |
| 66 (26.2%)                                       |
| Percent inhibition                               |
| 15.9±17.7                                       |
| 11.2±15.5                                       |
| Reactivity time (RT)                             |
| 5.0 (8)                                          |
| *2/**2 (n=10, 3.6%)                              |
| P                                                |
| CYP2C9 allele 2                                  |
| wt/wt (n=177, 63.7%)                             |
| wt/*2 (n=80, 28.8%)                              |
| *2/*2 (n=10, 3.6%)                               |
| P                                                |
| Base PRU                                         |
| 299.0±55.1                                       |
| PRU                                              |
| 301.4±57.0                                       |
| 265.9±78.7                                       |
| PRU <208                                         |
| 252.1±79.3                                       |
| 39 (22.0%)                                       |
| Percent inhibition                               |
| 14.7±17.2                                       |
| 18.0±18.7                                       |
| Reactivity time (RT)                             |
| 5.0 (9)                                          |
| *3/*3 (n=1, 0.3%)                                |
| P                                                |
| CYP2C19 allele 2                                 |
| CYP2C19 allele 3                                 |
| wt/wt (n=232, 83.5%)                             |
| wt/*3 (n=41, 14.8%)                              |
| *3/*3 (n=1, 0.3%)                                |
| P                                                |
| Base PRU                                         |
| 299.1±55.1                                       |
| PRU                                              |
| 291.5±50.7                                       |
| 262.8±79.1                                       |
| PRU <208                                         |
| 257.3±73.5                                       |
| 59 (25.4%)                                       |
| Percent inhibition                               |
| 15.6±17.8                                       |
| 14.4±16.1                                       |
| Reactivity time (RT)                             |
| 5.0 (8)                                          |
| *2/*2 (n=1, 0.3%)                                |
| P                                                |
| Base PRU                                         |
| 298.9±54.2                                       |
| PRU                                              |
| 299.0±59.6                                       |
| 251.9±76.1                                       |
| PRU <208                                         |
| 285.3±81.3                                       |
| 50 (24.9%)                                       |
| Percent inhibition                               |
| 17.7±18.4                                       |
| 10.8±14.9                                       |
| Reactivity time (RT)                             |
| 5.0 (9)                                          |
| *2/*2 (n=1, 0.3%)                                |
| P                                                |
| Base PRU                                         |
| 297.2±57.1                                       |
| PRU                                              |
| 299.9±53.3                                       |
| 261.7±81.8                                       |
| PRU <208                                         |
| 259.3±71.5                                       |
| 49 (28.5%)                                       |
| Percent inhibition                               |
| 16.0±17.5                                       |
| 15.2±17.8                                       |
| Reactivity time (RT)                             |
| 6.0 (8)                                          |
| *2/*2 (n=1, 0.3%)                                |
| P                                                |
| Base PRU                                         |
| 298.0±55.8                                       |
| PRU                                              |
| 299.8±56.0                                       |
| 263.1±75.4                                       |
| PRU <208                                         |
| 254.3±79.5                                       |
| 30 (24.8%)                                       |
| Percent inhibition                               |
| 15.2±17.3                                       |
| 17.2±18.5                                       |
| Reactivity time (RT)                             |
| 4.0 (8)                                          |
| *2/*2 (n=1, 0.3%)                                |
| P                                                |

Quantitative variables are presented as means: standard deviation or median (IR) and categorical variables as frequency and percentage. PRU - platelet reactivity units; RT - reactivity time between first dose of clopidogrel and determination of reactivity, median of days (interquartile range); wt - wild type.
The relationship between diabetes mellitus and PRC is well known and has been previously documented (32–34) with a few exceptions (28, 35). In this line, Kang et al. (28) did not find that the presence of diabetes mellitus was an independent predictor of high platelet reactivity (OR=1.681, 95% CI 0.750–3.759). In our study, we did not observe an association between diabetes mellitus and PRC. We do not have an explanation for such a puzzling finding, but we speculate that the relatively small sample of our study and the one by Kang (28) and Park (35) might have played a role. In our study, patients with high platelet reactivity were older, had higher BMI, and had anemia, which is also in line with previous studies (2, 36, 37).

Although there is limited experience, investigators are evaluating a potential usefulness of the determination of certain polymorphisms by point-of-care systems to facilitate decisions regarding the optimal antiplatelet therapy in the initial phase of the ACS patient care. This might represent also a clinical scenario where the determination of these polymorphisms might help to take decisions in short periods of time. In this regard, pilot studies suggest that these determinations may help to identify patients who benefit from a second loading dose of clopidogrel or a more potent antiplatelet drug (38, 39).

Future studies will clarify whether genotyping may help to decide the optimal treatment in patients with ACS. Probably, as shown by our results, the future is not in the analysis of a single polymorphism but a panel of them, among which CYP3A4*1B could be taken into account. Finally, in the era of “supermachines,” with the development of technologically advanced and expensive techniques, information obtained from medical notes and simple blood tests might still be useful in the identification of a significant proportion of patients with poor response to clopidogrel.

**Study limitations**

Our study has a few limitation. First, the sample size was modest and was determined by the maximum number of patients recruited in the interval indicated. In second place, although unlikely, the low prevalence of the CYP3A4*1B allele may have had an impact on the findings. However, allelic prevalence was similar to other studies (13, 15) and it was in accordance with the Hardy–Weinberg equilibrium (13) and the number of variables in the adjusted model had a relationship greater than 10 respect to the dependent variable. Another potential limitation is the determination of platelet reactivity at only one time point. As strength, we note that is a study, which evaluates six SNPs as well as a set of clinical variables in a population of patients with ACS.

**Conclusion**

CYP3A4*1B polymorphism may be an independent determinant of poorer response to clopidogrel in patients with ACS, although the variability in response to clopidogrel explained by the six polymorphisms is poor when compared with clinical variables.
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