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ORIGINAL RESEARCH

Pharmacogenetic association study on clopidogrel response in Puerto Rican Hispanics with cardiovascular disease: a novel characterization of a Caribbean population

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Dagmar F Hernandez-Suarez,[1] Mariana R Botton,[2] Stuart A Scott,[2] Matthew I Tomey,[3] Mario J Garcia,[4] Jose Wiley,[4] Pedro A Villablanca,[5] Kyle Melin,[6] Angel Lopez-Candales,[7] Jessicca Y Renta,[8] Jorge Duconge[9]

[1]Cardiovascular Medicine Division, Department of Medicine, University of Puerto Rico School of Medicine, San Juan, PR, USA; [2]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [3]Cardiovascular Medicine Division, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [4]Division of Cardiovascular Diseases, Department of Medicine, Montefiore Medical Center/Albert Einstein College of Medicine New York, NY, USA; [5]Division of Cardiology, Department of Medicine, New York University Langone Medical Center, New York, NY, USA; [6]Department of Pharmacy Practice, University of Puerto Rico Medical Sciences Campus, San Juan, PR, USA; [7]Cardiovascular Medicine Division, Department of Medicine, University of Puerto Rico School of Medicine, San Juan, PR, USA; [8]Department of Biochemistry, University of Puerto Rico Medical Sciences Campus, San Juan, PR, USA; [9]Pharmaceutical Sciences Department, University of Puerto Rico Medical Sciences Campus, San Juan, PR, USA

Correspondence: Dagmar F Hernandez-Suarez
University of Puerto Rico School of Medicine, Medical Sciences Building, PO Box 365067, San Juan, PR 00936-5067, USA
Tel +1 787 758 2525
Fax +1 787 754 1739
Email dagmar.hernandez@upr.edu

Introduction: High on-treatment platelet reactivity (HTPR) to clopidogrel imparts an increased risk for ischemic events in adults with coronary artery disease. Platelet reactivity varies with ethnicity and is influenced by both clinical and genetic variables; however, no clopidogrel pharmacogenetic studies with Puerto Rican patients have been reported. Therefore, we sought to identify clinical and genetic determinants of on-treatment platelet reactivity in a cohort of Puerto Rican patients with cardiovascular disease.

Methods: We performed a retrospective study of 111 patients on 75 mg/day maintenance dose of clopidogrel. Patients were allocated into 2 groups: Group I, without HTPR; and Group II, with HTPR. Platelet function was measured ex vivo using the VerifyNow® P2Y12 assay and HTPR was defined as P2Y12 reaction units (PRU) ≥230. Genotyping testing was performed using Taqman® Genotyping Assays.

Results: The mean PRU across the cohort was 203±61 PRU (range 8–324), and 42 (38%) patients had HTPR. Multiple logistic regression showed that 27% of the total variation in PRU was explained by a history of diabetes mellitus, hematocrit, *CYP2C19*2*, and *PON1* p.Q192R. Body mass index (odds ratio [OR]=1.15; 95% CI: 1.03–1.27), diabetes mellitus (OR=3.46; 95% CI: 1.05–11.43), hematocrit (OR=0.75; 95% CI: 0.65–0.87), and *CYP2C19*2* (OR=4.44; 95% CI: 1.21–16.20) were the only independent predictors of HTPR.

Conclusion: Moreover, we propose a predictive model to determine PRU values as measured by VerifyNow P2Y12 assay for the Puerto Rican Hispanic population. This model has the potential to identify Hispanic patients at higher risk for adverse events on clopidogrel.

Keywords: clopidogrel, platelet reactivity, genotyping, Hispanics, Puerto Rico

Introduction

Clopidogrel is a platelet adenosine diphosphate (ADP) receptor inhibitor commonly used to prevent thrombotic events in patients with acute coronary syndrome (ACS), ischemic stroke, carotid artery stenosis (CAS), and peripheral artery disease (PAD). Clopidogrel remains one of the most widely prescribed ADP receptor blockers, used by up to 40,000,000 people and with previous reports of up to 9 billion dollars a year in global sales.[1,2] However, significant variability in clinical response and platelet inhibition has been observed, which can lead to decreased antithrombotic effect for some patients.[3] Multiple clinical, cellular, genetic, and pharmacokinetic factors have been suggested as determinants of this variability;[4] however, the evidence supporting these variables and their effect on platelet reactivity among patients on clopidogrel is still conflicting.[5] In addition, the importance of ancestry and ethnicity on clopidogrel

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responsiveness among patients of underrepresented minority populations is still uncertain and warrants further research.[6]

To date, most clopidogrel responsiveness studies have focused on the association between *CYP2C19* variant alleles (e.g., *CYP2C19*2* and **3*) and both high on-treatment platelet reactivity (HTPR) and adverse cardiovascular events.[7,8] In addition, *PON1* p.Q192R, *ABCB1* c.3435C>T, and the *P2RY12* H2 haplotype have also been proposed as contributors to adverse outcome risk on clopidogrel.[9] In contrast, the *CYP2C19*17* allele increases expression and enzyme activity, leading to enhanced platelet inhibition and a higher risk of bleeding.[10] However, only ~12% of the variability in clopidogrel response has been explained by *CYP2C19* alone, suggesting that other important clinical, genetic, or environmental factors have yet to be identified.[11–14]

Hispanics have a higher prevalence of cardiovascular risk factors, recurrence rate of thrombotic events, and worse cardiovascular outcomes when compared with non-Hispanic Caucasians; however, there is a paucity of antiplatelet studies reported on this group. As such, greater efforts should be directed to include this population in cardiovascular pharmacogenetic studies. Interestingly, among all Hispanic sub-groups living within the USA and its territories, Puerto Ricans are the only one with higher age-adjusted death rates when compared with non-Hispanic Whites.[15] Additionally, among Hispanic adults of diverse backgrounds, the prevalence of adverse cardiovascular disease risk profiles is higher among Puerto Ricans.[16] Moreover, our preliminary findings in Puerto Ricans suggest that interindividual variation in ancestral contributions have significant implications on clopidogrel responsiveness.[17,18] Hence, we hypothesized that due to the trihybrid admixture and high prevalence of cardiovascular risk factors, Puerto Ricans might have a unique clinical and genetic contribution to clopidogrel responsiveness that could change our current approach to antiplatelet therapy in this population.

Furthermore, clopidogrel is preferred among ADP receptor blockers in Puerto Rico, largely because of its availability as a generic drug and lower cost. Since genetic determinants of impaired response to clopidogrel are not currently known in Caribbean Hispanics, we sought to determine pharmacogenetic variants associated with platelet reactivity to clopidogrel in Puerto Rican Hispanic patients.

Methods

Study design and ethics

This was a multicenter case–control study of Puerto Rican Hispanics patients receiving antiplatelet therapy who were recruited between January and February 2017. The Human Research Subjects Protection Office (HRSPO) approved this study (Protocol No. A4070416). HRSPO serves as the administrative office for the University of Puerto Rico Medical Science Campus Institutional Review Boards (assurance #FWA00005561). The protocol was also conducted in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice. All patients provided written informed consent.

Patient population and data collection

A total of 111 patients of Hispanic Puerto Rican descent on clopidogrel therapy were consecutively recruited at the University District Hospital and the Cardiovascular Center of Puerto Rico and the Caribbean, in San Juan, PR. Past medical history and preadmission laboratory data were obtained from medical record. Puerto Rican Hispanics aged ≥21 years on 75 mg/day maintenance dose of clopidogrel for at least 7 consecutive days were included in the study. Exclusion criteria included the use of any oral anticoagulant or glycoprotein IIb/IIIa receptor inhibitors, administration of other ADP receptor blocker other than clopidogrel within 2 weeks of enrollment, hematocrit (Hct) ≤25%, platelet count $<100\times10^9$/L, blood urine nitrogen (BUN)/creatinine >30/1.5 mg/dL, known platelet function disorder, or active hepatic disease. Patients were allocated into 2 groups based on P2Y12 reaction units (PRU) cutoff values:[7,19] Group I without HTPR (PRU<230) and Group II with HTPR (PRU≥230).

Platelet function testing

Blood samples for platelet function and genetic testing were collected within 2 days of a scheduled vascular/cardiac minimally invasive procedure during the preadmission evaluation. Whole blood was drawn from a peripheral vein through a 22-gauge needle. An initial 2 mL sample was collected from each participant and saved for other laboratory tests as part of the preadmission process. A second tube containing 3.2% sodium citrate was obtained with 2 mL blood for platelet function testing. Platelet function was measured *ex vivo* using the US Food and Drug Administration-approved point-of-care VerifyNow® P2Y12 analyzer following the manufacturer's instructions (Accumetrics, Inc., San Diego, CA, USA).

Since the definition of HTPR in the Hispanic population is unknown, we used the upper tertile PRU value (≥230) previously obtained by our group in a pilot study of Puerto Rican patients on clopidogrel therapy to define HTPR.[20] This value has also been used to define HTPR in other studies[7,19] and is similar to other cutoffs reported in literature.[21,22] In addition, a PRU value of 230 has been recognized by the manufacturer as the cutoff for samples with no platelet inhibition.

DNA extraction and genotyping

Genomic DNA was extracted using the QIAamp DNA Blood Maxi Kit (spin protocol; QIAGEN Inc., Venlo, Limburg) according to the manufacturer's instructions and genotyped for a panel of candidate variants using TaqMan® SNP Genotyping Assay Reagent kits (Applied Biosystems, Carlsbad, CA, USA): *CYP2C19*2, *3, *4* and **17*; *ABCB1* c.3435C>T; *PON1* p.Q192R; *P2RY12* H2 haplotype; *B4GALT2* c.909C>T, and c.366G>C variants; *CES1* c.428G>A and *PEAR1* rs12041331 and rs2768759.[23]

Statistical analysis

Continuous variables were compared using the 2-tailed Student's *t*-test while Chi-square or Fisher's exact tests were used for categorical data as appropriate. The Hardy–Weinberg equilibrium (HWE) test was applied as a quality control for genotyping; deviation from HWE was estimated using a χ^2 goodness-of-fit test with 1° of freedom. Comparison of minor allele frequencies (MAFs) between our cohort and reference populations were performed using a *Z*-test for population proportions. Simple linear regression analysis was performed to determine the association between all measurements and platelet reactivity. A multivariate linear regression was used to identify the contribution of all significant variables to platelet reactivity variability, and a multiple logistic regression was performed to determine predictors of HTPR. The corresponding odds ratio (OR) and 95% CI were calculated. Statistical analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA), and p-values<0.05 were considered statistically significant.

Results

Study population

All the study participants (n=111) underwent platelet function testing and genotyping. Enrolled individuals were distributed into the non-HTPR (n=69, 62%) and HTPR (n=42, 38%) groups. The indications for the patient cohort included coronary artery disease (CAD; 77%), PAD (34%), CAS (7%), cerebral artery aneurysm (2%), and stroke (2%). All patients were on 75 mg/day maintenance dose of clopidogrel for >7 days. The patient baseline characteristics are detailed in Table 1. HTPR patients had higher body mass index (BMI), prevalence of diabetes mellitus (DM), use of proton-pump inhibitors (PPI), and calcium channel blockers (CCB), but lower hemoglobin and Hct levels compared with non-HTPR patients.

Genotyping

The genotyping results are illustrated in Tables 2 and 3. Approximately 25% of patients carried at least 1 copy of the *CYP2C19*2* allele, which was the only variant with different allele frequencies between groups. Due to the low frequency of homozygous variant allele carriers for most of the tested genes (e.g., *CYP2C19*2/*2*), carriers of at least 1 variant allele were compared with wild-type individuals (Table 3). Notably, the non-HTPR group had a higher proportion of patients with at least 1 copy of the *PON1* p.Q192R variant.

The MAFs of *CYP2C19*2*, *CYP2C19*17*, *ABCB1* 3435C>T, *P2RY12* H2, *B4GALT2* 909C>T, *B4GALT2* 366G>C, *PEAR1* rs12041331, and *PON1* p.Q192R in the study cohort were 15%, 14%, 37%, 8%, 6%, 3%, 20%, and 47%, respectively. We also compared all the MAFs observed in the study population with those from reference populations (data taken from the 1000 Genomes Phase I project).[24] We found no significant differences in MAFs between our cohort and the Puerto Ricans sample included in the 1000 Genomes Phase I project (p>0.05). No significant departure from HWE was detected for the tested variants except for *CYP2C19*2* (Table 2), which was attributed to the relatively small sample size of the cohort.

Determinants of platelet reactivity

The distribution of platelet reactivity in the study cohort is illustrated in Figure 1. The mean platelet reactivity to clopidogrel was 203±61 PRU (range: 8–324). Five clinical factors (age, history of DM, Hct, BUN, use of CCB) and 2 genetic variants (*CYP2C19*2, PON1* p.Q192R) were associated with platelet reactivity by linear regression (Table 4). Notably, Hct and *PON1* p.Q192R were negatively correlated with platelet reactivity. Although not significant, smoking had a tendency to decrease PRU values while the use of PPI had the opposite effect (Figure 2). All the factors associated with platelet reactivity, as well as BMI and PPI (Table 4), were included in a multiple linear regression analysis. DM, Hct, *CYP2C19*2,* and *PON1* p.Q192R were the only independent predictors of platelet reactivity after adjusting for all possible confounders and interactions. The contribution of the studied genetic variants to platelet reactivity was ~6%; however, the model increased to 34% when clinical variables were added (Table 5).

Table 1 Baseline clinical characteristics of the study patients according to on-treatment platelet reactivity

Characteristics[a]	All (n=111)	Non-HTPR (n=69)	HTPR (n=42)	*p*-value
Age (years)	69±11	68±11	71±11	0.20
Male gender	54 (49)	37 (54)	17 (41)	0.18
BMI (kg/m^2)	28±6	27±6	30±6	0.01
Risk factors				
Hypertension	100 (90)	61 (88)	39 (93)	0.53
Diabetes mellitus	67 (60)	36 (52)	31 (74)	0.02
Dyslipidemia	95 (86)	58 (84)	37 (88)	0.56
Active smoker	16 (14)	13 (19)	3 (7)	0.10
Main vascular diagnosis				
Coronary artery disease	85 (77)	55 (80)	30 (71)	0.46
Peripheral artery disease	38 (34)	20 (29)	18 (43)	
Carotid stenosis	8 (7)	6 (9)	2 (5)	
Stroke	2 (2)	1 (1)	1 (2)	
Cerebral aneurysm	2 (2)	1 (1)	1 (2)	
Laboratory data				
WBC (×10^3/μL)	8.0±2.2	8.0±2.3	8.0±2.0	0.90
Hgb (g/dL)	13.3±1.6	13.9±1.4	12.4±1.7	<0.01
Hct (%)	39.5±4.7	40.0±3.9	37.1±4.9	<0.01
Platelet count (×10^3/μL)	237±70	239±71	235±68	0.80
BUN (mg/dL)	19.9±7.7	18.3±7.8	22.6±7.0	<0.01
Creatinine (mg/dL)	0.9±0.3	0.9±0.3	1.0±0.3	0.23
Concomitant therapy				
Aspirin	72 (65)	45 (65)	27 (64)	0.92
Proton-pump inhibitors	22 (20)	9 (13)	13 (31)	0.02
Statins	86 (77)	51 (74)	35 (83)	0.25
Calcium channel blockers	30 (27)	13 (19)	17 (40)	0.01
Cilostazol	24 (22)	17 (25)	7 (17)	0.32

Note: [a]Values are mean ± SD or n (%).

Abbreviations: BMI, Body mass index; BUN, blood urine nitrogen; Hct, hematocrit; Hgb, hemoglobin; HTPR, high on-treatment platelet reactivity; WBC, white blood count.

Development of a predictive model for platelet reactivity

An initial predictive model to estimate platelet reactivity was obtained from the 9 variables included in the multiple linear regression analysis. This initial model estimates PRU by the following equation:

Initial model (R^2=34%, p<0.0001):

PRU=208+0.8(age)+1.6(BMI)+30.0(DM)−3.4(Hct)+0.8(BUN)+15.7(PPI)+12.6(CCB)+24.0(*CYP2C19*2*)−24.6(*PON1* p.Q192R)

where age is in years, BMI in kg/m^2, Hct in %, BUN in mg/dL, DM, PPI, and CCB are coded as 0 if absent and 1 if present. The *CYP2C19*2* and *PON1* p.Q192R variants are coded as 0 for homozygous wild-type genotypes and 1 if at least 1 copy of the variant allele is carried.

Since clinical genotyping may not be feasible in some medical settings, particularly in low-resource institutions, we designed a clinical model to be considered in the case of unavailable genetic data. To obtain this model, a multiple linear regression analysis was first performed excluding the genetic data. BMI, DM, and Hct were the only clinical variables that predicted platelet reactivity and, therefore, were included in the following model:

Clinical model (R^2=23%, p<0.0001):

PRU=308+2.0(BMI)+31.5(DM)−4.6(Hct)

Finally, a final model was obtained from the 4 clinical and genetic variables that independently predicted platelet reactivity:

Final model (R^2=27%, p<0.0001):

PRU=348+38.1(DM)−3.9(Hct)+23.4(*CYP2C19*2*)−29.1(*PON1* p.Q192R)

Model diagnostic analysis is shown in Figure 3. As seen, the final model has a normal probability plot for residuals and a good visual correlation between the observed versus fitted PRU values.

Table 2 Genotype frequencies of all polymorphisms of interest in the studied groups and their corresponding *p*-values after comparison between HTPR and non-HTPR

Genetic variants and genotypes[a]	All (n=111)	Non-HTPR (n=69)	HTPR (n=42)	*p*-value	HWE test
*CYP2C19*2* (c.681G>A) (rs4244285)					
GG	83 (75)	57 (83)	26 (62)	0.02	0.01
GA	22 (20)	8 (11)	14 (33)		
AA	6 (5)	4 (6)	2 (5)		
*CYP2C19*17* (c.806C>T) (rs12248560)					
CC	81 (73)	50 (72)	31 (74)	0.77	0.81
CT	28 (25)	17 (25)	11 (26)		
TT	2 (2)	2 (3)	0 (0)		
ABCB1 (c.3435C>T) (rs1045642)					
CC	42 (38)	24 (35)	18 (43)	0.55	0.54
CT	55 (49)	37 (54)	18 (43)		
TT	14 (13)	8 (11)	6 (14)		
PON 1 (c.575A>G [p.Q192R]) (rs662)					
AA	32 (29)	15 (22)	17 (40)	0.09	0.66
AG	53 (48)	35 (51)	18 (43)		
GG	26 (23)	19 (27)	7 (17)		
P2RY12 (c.744C>T) (rs2046934)					
CC	93 (84)	59 (86)	34 (81)	0.53	0.35
CT	18 (16)	10 (14)	8 (19)		
TT	0 (0)	0 (0)	0 (0)		
B4GALT2 (c.909C>T) (rs1061781)					
CC	98 (88)	60 (87)	38 (90)	0.76	0.51
CT	13 (12)	9 (13)	4 (10)		
TT	0 (0)	0 (0)	0 (0)		
B4GALT2 (c.366G>C) (rs1859728)					
GG	104 (94)	65 (94)	39 (93)	1.00	0.73
GC	7 (6)	4 (6)	3 (7)		
CC	0 (0)	0 (0)	0 (0)		
CES1 (c.428G>A) (rs71647871)					
GG	109 (98)	68 (99)	41 (98)	1.00	0.92
GA	2 (2)	1 (1)	1 (2)		
AA	0 (0)	0 (0)	0 (0)		
PEAR1 (rs12041331)					
GG	70 (66)	44 (67)	26 (65)	0.05	0.26
GA	30 (28)	19 (29)	11 (27)		
AA	6 (6)	3 (4)	3 (8)		
PEAR1 (rs2768759)					
AA	30 (28)	21 (32)	9 (22)	0.48	0.33
AC	48 (45)	27 (42)	21 (51)		
CC	28 (27)	17 (26)	11 (27)		

Notes: Departure from the HWE is also shown (*p*-values in this column correspond to the χ^2 goodness-of-fit test, with 1° of freedom). [a]Values are represented in n (%). Alleles *CYP2C19*3* and *CYP2C19*4* were not observed in the study population.
Abbreviations: HTPR, high on-treatment platelet reactivity, HWE, Hardy–Weinberg equilibrium.

Predictors of HTPR phenotype

Clinical and genetic characteristics associated with HTPR are presented in Table 6. DM, BMI, Hct, BUN, PPI, CCB, *CYP2C19*2,* and *PON1* p.Q192R were all correlated with HTPR. Interestingly, Hct and *PON1* p.Q192R had a protective effect on determining HTPR. When all these variables were included in a multivariable logistic regression analysis, we found that BMI (OR=1.15; 95% CI: 1.03–1.27), DM (OR=3.46; 95% CI: 1.05–11.43), Hct (OR=0.75; 95% CI: 0.65–0.87), and *CYP2C19*2* (OR=4.44; 95% CI: 1.21–16.20) were the only factors independently correlated with PRU≥230.

Discussion

The paucity of reports on antiplatelet response in the Hispanic population and recent reports linking ethnicity with

Table 3 Genotype frequency of carriers of at least 1 variant allele

Genetic variants and genotypes[a]	All (n=111)	Non-HTPR (n=69)	HTPR (n=42)	*p*-value
*CYP2C19*2* (c.681G>A) (rs4244285)				
Wild type	83 (75)	57 (83)	26 (62)	0.01
Carrier[b]	28 (25)	12 (17)	16 (38)	
*CYP2C19*17* (c.806C>T) (rs12248560)				
Wild type	81 (73)	50 (72)	31 (74)	0.88
Carrier	30 (27)	19 (28)	11 (26)	
ABCB1 (c.3435C>T) (rs1045642)				
Wildtype	42 (38)	24 (35)	18 (43)	0.39
Carrier	69 (62)	45 (65)	24 (57)	
PON1 (c.575A>G [p.Q192R]) (rs662)				
Wild type	32 (29)	15 (22)	17 (40)	0.03
Carrier	79 (71)	54 (78)	25 (60)	
PEAR1 (rs12041331)				
Wild type	70 (66)	44 (67)	26 (65)	0.86
Carrier	36 (34)	22 (33)	14 (35)	
PEAR1 (rs2768759)				
Wild type	30 (28)	21 (32)	9 (22)	0.25
Carrier	76 (72)	44 (68)	32 (78)	

Notes: [a]Values are represented in n (%). [b]Carrier includes genotypes with at least 1 copy of the mutant allele. Alleles *CYP2C19*3* and *CYP2C19*4* were not observed in the study population. *P2RY12*, *B4GALT2*, and *CES1* variants were not included as no homozygotes for the mutant allele were reported.
Abbreviation: HTPR, high on-treatment platelet reactivity.

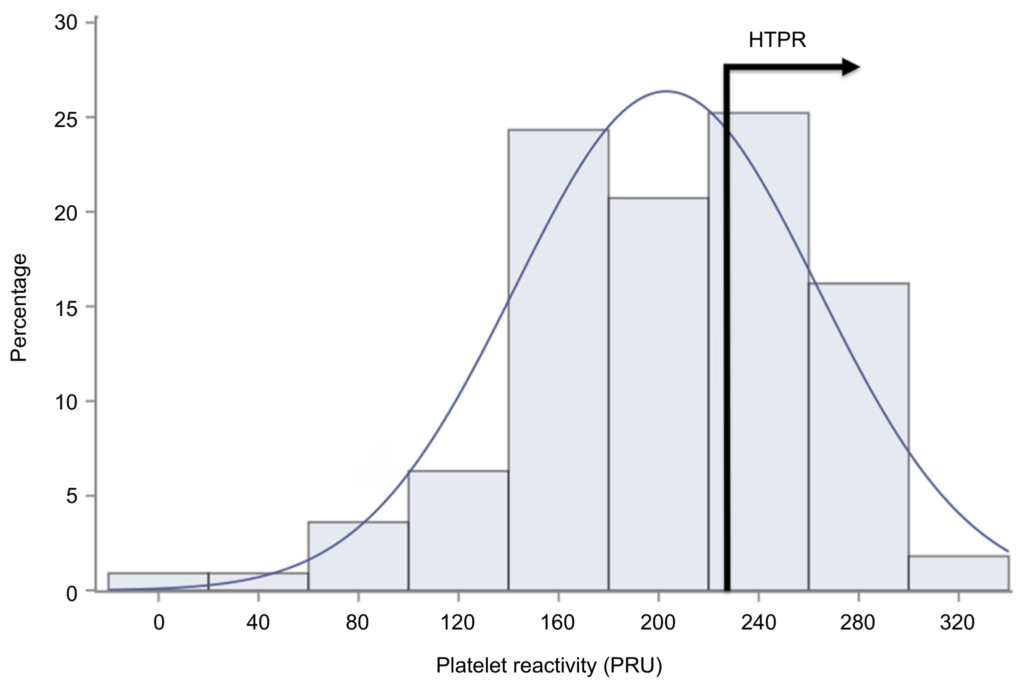

Figure 1 Distribution of platelet reactivity as measured by PRU.
Abbreviations: HTPR, high on-treatment platelet reactivity; PRU, P2Y12 reaction units.

platelet reactivity[25] prompted our study to identify clinical and genetic determinants of on-treatment platelet reactivity among cardiovascular Puerto Rican patients on clopidogrel. To the best of our knowledge, no studies on clopidogrel pharmacogenetics in Caribbean Hispanics have previously been reported. Our study determined that DM, Hct, *CYP2C19*2,* and *PON1* p.Q192R were predictors of platelet reactivity on clopidogrel in Puerto Rican patients. In addition, we developed a novel multivariable model to predict PRU (measured by VerifyNow-P2PY12) in the Hispanic population.

Table 4 Simple linear regression between important variables and platelet reactivity

Characteristics[a]	Estimate coefficient	Standard error	t	*p*-value
Age	1.37	0.50	2.73	<0.01
Male gender	−11.34	11.50	−0.99	0.33
BMI	1.83	0.95	1.94	0.06
Diabetes mellitus	37.26	11.25	3.31	<0.01
Active smoker	−22.22	16.29	−1.36	0.18
WBC	3.3	2.8	1.19	0.24
Hct	−4.8	1.2	−3.96	<0.01
Plat	−0.1	0.1	0.72	0.47
BUN	2.7	0.7	3.63	<0.01
Creatinine	20.9	19.8	1.06	0.29
PPI	24.90	14.28	0.08	0.08
CCB	33.55	12.60	2.66	<0.01
Cilostazol	−15.2	14.0	−1.09	0.28
*CYP2C19*2*	27.40	13.03	2.10	0.04
*CYP2C19*17*	−2.40	12.99	−0.18	0.85
ABCB1 c.3435C>T	−12.70	11.84	−1.07	0.29
PON1 p.Q192R	−26.19	12.50	−2.10	0.04
P2RY12 c.744C>T	15.55	15.59	1.00	0.32
B4GALT2 c.909C>T	−27.80	17.75	−1.57	0.12
B4GALT2 c.366G>C	−26.62	23.61	−1.13	0.26
PEAR1 rs12041331	4.71	12.06	0.39	0.70
PEAR1 rs2768759	2.83	12.88	0.22	0.83

Note: [a]CES1 was not included due to the observed low frequency (n=2).
Abbreviations: BMI, body mass index; BUN, blood urine nitrogen; CCB, calcium channel blockers; Hct, hematocrit; PPI, proton-pump inhibitors.

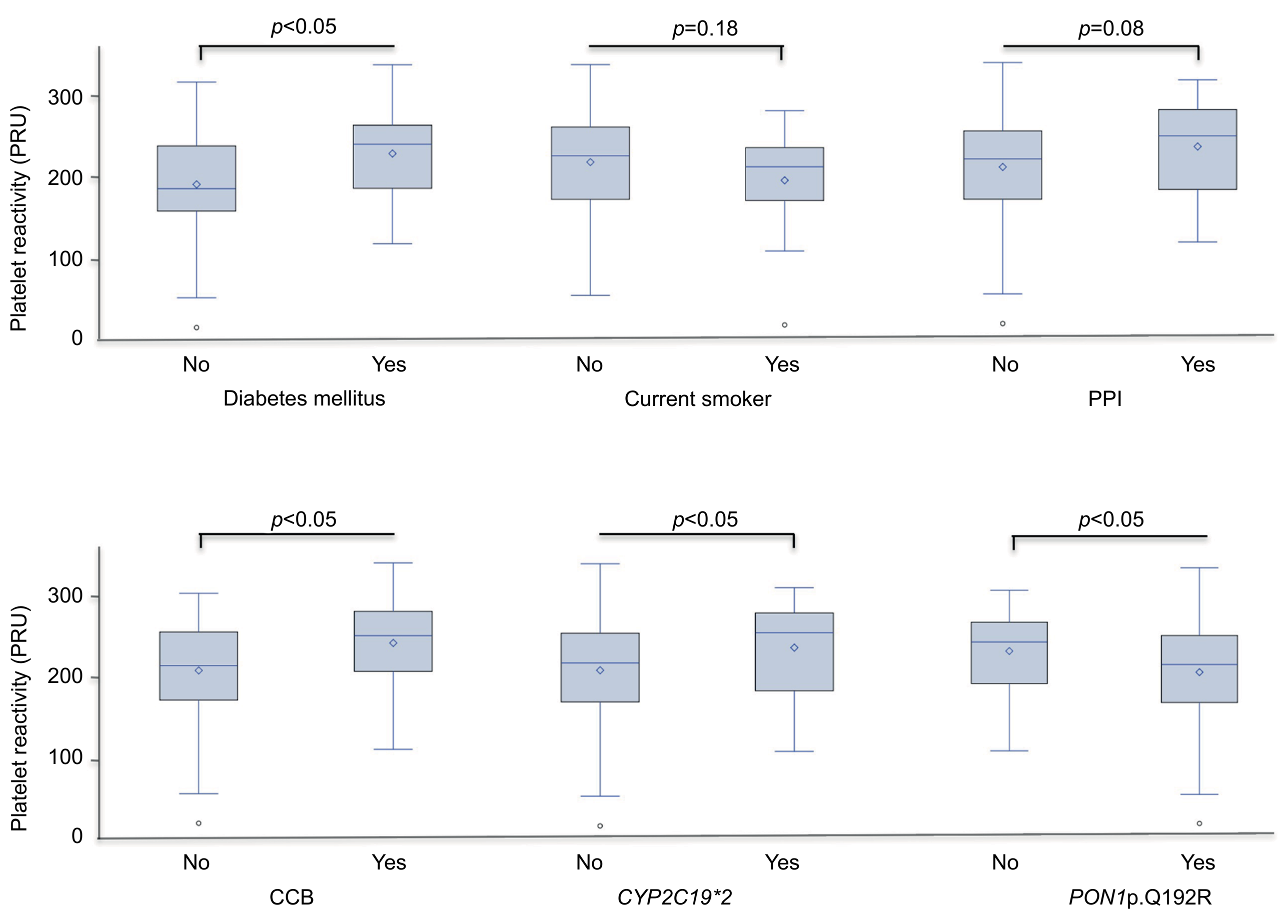

Figure 2 Association between important clinical and genetic characteristics with platelet reactivity.
Abbreviations: CCB, calcium channel blockers; PPI, proton-pump inhibitors; PRU, P2Y12 reaction units.

In the present study, 38% of our patient population had HTPR defined as a PRU≥230, which was generally consistent with data from Mallouk et al, indicating that between 16% and 50% of patients treated with clopidogrel had a poor biological response.[26] However, comparisons of HTPR prevalence across different populations is challenging as HTPR depends on the cutoff value used. Furthermore, on-treatment platelet reactivity is multifactorial, as it is influenced by clinical and genetic factors, time and method of platelet reactivity assessment, antiplatelet indication, ancestry, and potentially other unknown variables.[10,27]

Puerto Rican Hispanics are unique as a result of their 500-year history of genomic admixture,[28] yet little is known about the frequencies of important pharmacogenetic variants in this population. Prior reports have determined the frequencies of *CYP2C19*2* and **3* in Puerto Ricans;[29,30] however, no information regarding other *CYP2C19* polymorphisms, clopidogrel pharmacogenes and their haplotype structure is currently known. We report the MAFs of 6 important clopidogrel pharmacogenes (*CYP2C19, ABCB1, PON1*, *P2RY12, B4GALT2, CES1,* and *PEAR1*) in a cohort of cardiovascular patients from the Commonwealth of Puerto Rico.

Table 5 Contribution of study variables to the interindividual variability in on-treatment platelet reactivity

	R^2_{adj} (%)	*p*-value
Genetic variables[a]	6	<0.0001
Clinical variables[b]	28	<0.0001
Clinical + genetic variables	34	<0.0001

Notes: [a]*CYP2C19*2* and *PON1* p.Q192R. [b]7 clinical variables (5 variables associated with platelet reactivity in Table 4, body mass index and proton-pump inhibitor).

A recent review of *CYP2C19* studies worldwide estimated a *CYP2C19*2* frequency between 9% in North Africans and 61% in Native Oceanians.[31] American admixed population had a frequency of 12%, which is similar to the 15% found in our study. Furthermore, we found no significant difference when the *CYP2C19*2* frequency in our cohort was compared with that in the Puerto Rican population included in the 1000 Genomes Project (13%).[24] In contrast, neither the *CYP2C19*3* nor the *CYP2C19*4* variants were identified in our cohort. This is consistent with previous reports in the Puerto Rican population.[29,30] In addition, the *CYP2C19*4* variant is very rare in the admixed American population.[31]

The *CYP2C19, PON1, P2RY12, ABCB1, CES1, PEAR1,* and, most recently, *B4GALT2* genes have been previously linked to clopidogrel response.[8,10,32–34] To date, most studies have focused on the association between *CYP2C19* and both HTPR and major adverse cardiovascular events.[35–37] Likewise, *PON1* p.Q192R, *ABCB1* c.3435C>T, *P2RY12* H2 haplotype, and *PEAR1* rs12041331 have been proposed to be related to the same outcomes. In contrast, the increased function *CYP2C19*17* allele results in greater enzyme expression and activity, leading to an enhanced platelet inhibition and increased risk of bleeding. Furthermore, low on-treatment platelet reactivity, defined as PRU<50 has been recently associated with the *B4GALT2* c.909C>T and c.366G>C variants in populations of European descendent.[10] In our study, no significant associations were found between these *B4GALT2* variants and platelet reactivity. The MAFs observed in the present cohort are lower than the frequencies previously reported in a cohort of individuals with mostly European

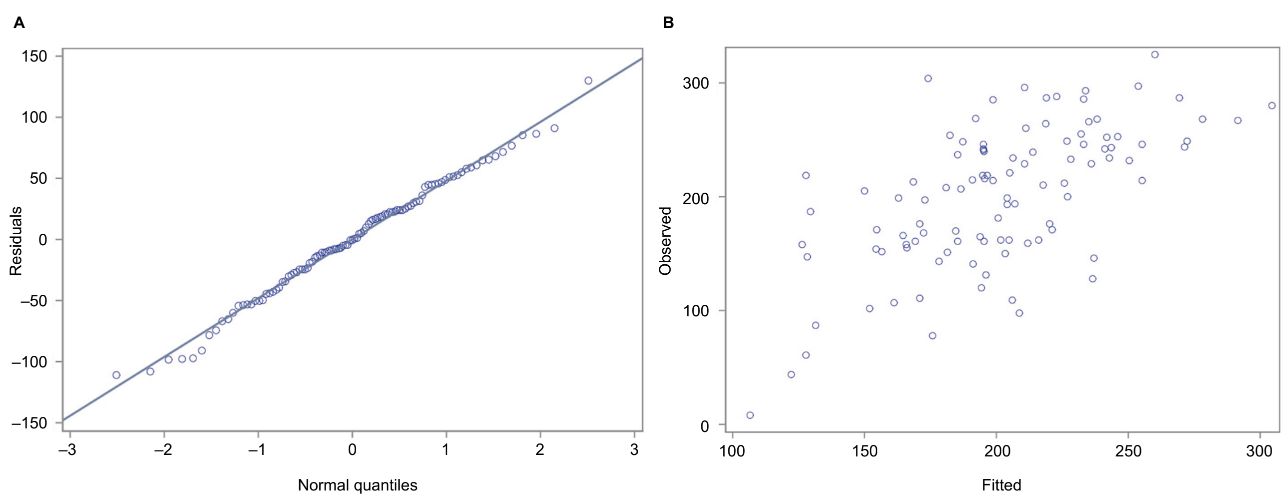

Figure 3 Model diagnostics analysis.
Notes: (**A**) Q-Q plot for normality analysis of residuals showing a normal distribution. (**B**) Observed versus fitted PRU values showing a strong visual correlation.
Abbreviation: PRU, P2Y12 reaction units.

Table 6 Stepwise logistic regression analysis to determine the best predictor of high on-treatment platelet reactivity

Variables	OR	95% CI	*p*-value	OR adjusted	95% CI	*p*-value
BMI	1.09	1.02–1.17	0.02	1.15	1.03–1.27	<0.01
Diabetes mellitus	2.58	1.12–5.95	0.03	3.46	1.05–11.43	0.04
Hct	0.79	0.70–0.90	<0.01	0.75	0.65–0.87	<0.01
PPI	2.99	1.15–7.79	0.03	2.41	0.66–8.89	0.18
CCB	2.93	1.24–6.94	0.01	1.81	0.56–5.90	0.33
*CYP2C19*2*	2.92	1.21–7.05	0.02	4.44	1.21–16.20	0.02
PON1 p.Q192R	0.41	0.18–0.95	0.04	0.32	0.10–1.04	0.06

Abbreviations: BMI, body mass index; CCB, calcium channel blockers; Hct, hematocrit; OR, odd ratios; PPI, proton-pump inhibitors.

ancestry (0.06 versus 0.10 for 909C>T and 0.03 versus 0.07 for 366G>C).[10] Also, *B4GALT2* 909C>T and 366G>C polymorphisms were in strong linkage disequilibrium ($p<0.001$; $D'=1.0$; $r^2=0.523$), which is expected for younger populations. The unique genetic background of Puerto Ricans might have contributed to this apparent discrepancy in the results. Thus, our data suggest that the association between these *B4GALT2* variants with on-treatment platelet reactivity is likely population-specific.

Only ~12% of the variability in clopidogrel response is explained by *CYP2C19* alone;[38] however, heritability estimates suggest that genetics can account for up to 73% of such variance.[11] In addition, these genes are involved in the bioactivation and metabolism of a large number of prescription drugs other than clopidogrel as well as drug classes, including antidepressants, benzodiazepines, mephenytoin, and PPI. Hence, the importance to study these genetic polymorphisms is not limited to antiplatelet drugs. Moreover, several non-genetic factors have been identified as possible determinants of poor biological response to clopidogrel, such as BMI, DM, concomitant use of some drugs, smoking status, Hct, and ethnicity.[13,25,39,40]

In the Caribbean population, the effects of clinical and genetic factors on clopidogrel responsiveness have not been fully elucidated. In our study, we found that DM, Hct, *CYP2C19*2,* and *PON1* p.Q192R were the only independent predictors of interindividual variability in platelet reactivity as measure by VerifyNow. Together, these variables explained 27% of PRU variability. In a similar study, Larsen et al, reported 21% of PRU variability determined by >10 clinical and genetic factors for a New Zealand ACS population.[41] Interestingly, both Hct and *PON1* p.Q192R were inversely correlated with platelet reactivity to clopidogrel in our cohort. The negative relationship between Hct levels and clopidogrel response has been previously demonstrated;[42] however, a correction of PRU for Hct lacks of clinical validity and does not improve the prediction of adverse cardiovascular events.[43]

The particular case of *PON1* p.Q192R is more controversial. In 2011, Bouman et al, tested the clinical relevance of *PON1* p.Q192R in patients undergoing percutaneous coronary intervention on clopidogrel treatment.[44] They found that p.192Q was associated with a reduced conversion of 2-oxo-clopidogrel to the active thiol metabolite when compared with the *PON1* p.192R variant. Consequently, p.Q192R resulted in a more efficient clopidogrel bioactivation. Moreover, p.192QQ homozygous individuals were at higher risk of stent thrombosis. Park et al, reported similar results in the CROSS-VERIFY Cohort study, where 1336 patients were genotyped and p.192Q was found to be an independent predictor of worse cardiovascular outcome and significantly associated with higher levels of small dense low-density lipoprotein cholesterol.[45] However, subsequent studies have failed to demonstrate a clear association between *PON1* and clopidogrel response variability.[46] Our study supports a positive effect of *PON1* on clopidogrel response in Puerto Ricans. In this population, patients with at least 1 copy of p.192R had a better response to clopidogrel, which underscores the importance of ancestry and admixture in determine the ultimate effect of pharmacogenetic variants.

Patients with DM have increased platelet reactivity and a higher percent of circulating immature platelets,[13,47] which could reduce the inhibitory effect of clopidogrel on platelet aggregation. Moreover, the loss of responsiveness to insulin in DM patients could lead to a reduced response to antiplatelet drugs, resulting in heightened platelet reactivity.[48] In fact, a recent study found an association between insulin receptor substrate-1 variants and HTPR with clopidogrel therapy in CAD patients with DM.[49] In Puerto Ricans, the effect of this interaction may be more prominent as the prevalence of DM is much higher when compared with other ethnic groups.[50,51]

The other genotyped variants included in our study (*CYP2C19*17*, *ABCB1*, *P2RY12, B4GALT2, CES1,* and *PEAR1*) were not found to be significantly associated with platelet reactivity in the Puerto Rican population. Similarly, BMI, use of PPI and CCB, or being a smoker did not predict platelet reactivity after multivariate analysis was performed. Current evidence is inconsistent about whether these factors determine clopidogrel responsiveness.[9,32,52–57]

HTPR is an objective measure of clopidogrel responsiveness and it has been associated with increased risk of adverse cardiovascular events.[7,35] In our sample, BMI, DM, Hct, and *CYP2C19*2* were predictors of PRU ≥230. Interestingly, we failed to find a significant association between *PON1* p.Q192R and HTPR after multivariate analysis. Given a lack of consensus regarding the best cutoff PRU value to define HTPR and since a PRU≥230 might not be the true threshold value for Puerto Ricans, we constructed a PRU predictive model able to provide an objective quantitative measurement of platelet reactivity to clopidogrel. Although other authors have proposed similar models,[42] none has been developed particularly for Hispanics and very few have considered patients with diagnosis other than coronary disease. Our study is novel as it offers clinicians a valuable clinical decision-support tool by using clinical and genetic information or clinical data alone, so they can identify patients at higher risk of having poor response to clopidogrel and thus facilitate prompt therapy optimization to minimize adverse cardiovascular events. Yet, further validation remains pending and the benefit of adding new variables still needs to be assessed. However, the proposed model is useful for a wide range of cardiovascular entities.

Some limitations of this study need to be highlighted. First, despite the relatively small sample, the study was adequately powered to discover strong risk predictors of HTPR with OR ≥3.0 for the homozygous risk genotype. However, weaker predictors conferring smaller risk increases may have remained undiscovered. Yet, this study was primarily designed to perform preliminary assessments on clopidogrel response in Puerto Rican Hispanics as a way to set the appropriate context for further pharmacogenetic studies in this population at large. Second, medication compliance was self-reported and, therefore, it is possible that some patients were not adherent to clopidogrel therapy. Finally, PRU was measured at a single time in each participant. Hence, intraindividual variability was not previously assessed. This could be important since repeat PRU measurements might detect important changes in clopidogrel responsiveness and help differentiate patients with and without adverse clinical events.[58,59]

Conclusion

In a representative sample of Puerto Rican patients with cardiovascular disease, DM, Hct, *CYP2C19*2,* and *PON1* p.Q192R were associated with on-treatment platelet reactivity. Additionally, we developed a predictive model to determine PRU values as measured by VerifyNow P2Y12 assay for the Puerto Rican Hispanic population. This model may be critical in identifying Hispanic patients at higher risk for adverse events on clopidogrel.

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Disclosure

The authors report no conflicts of interest in this work.

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