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Association of the C3435T Multi-Drug Resistance Gene-1 (*MDR-1*) Polymorphism with Clopidogrel Resistance among Moroccan Acute Coronary Syndromes (ACS) Patients

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Abstract

Background: Antiplatelet drugs are recommended as one of the main medications used in Acute Coronary Syndromes (ACS) patients. However, an inter-individual variability in platelet response to Clopidogrel has been found in a substantial group of them. Genetic is known be the dominant influencing factor for individual and inter-ethnic variations in drug responses. Polymorphisms in the genes involved in Clopidogrel absorption, biotransformation to the active metabolite, or platelet response to adenosine diphosphate (ADP) have been associated to this impaired response. Interestingly, Multi-Drug Resistance gene-1 (*MDR-1*) polymorphism influences oral bioavailability of clopidogrel and prognosis of ACS patients. The objectives of our study are, first, to determine the frequency of the C3435T *MDR1* polymorphism among Moroccan ACS patients compared to healthy subjects; and second, to assess its effect on Clopidogrel response in a sample of Moroccan ASC patients.

Methods and results: 40 ACS patients were recruited and compared to 99 healthy controls. Extracted DNA samples were genotyped by PCR-RFLP method using MboI restriction enzyme. The VerifyNow assay was used to evaluate platelet function among ACS patients. Our results showed that HTA, Smoking, Creatinine and Sex were statistically associated to Clopidogrel resistance (P=0.05; P=0.05; P=0.05 and P=0.04 respectively). 63.64% of the ST (+) patients were carrying the mutant allele, 54.5% of them having the heterozygous genotype and 36.4% the homozygous mutant one, compared to 9.1% having the homozygous wild type genotype. 62.5% of the resistant group was carrying the mutant allele (50% of them had the TT mutant genotype, 25% CT and 25% CC profiles). Among cases, 42.5% were homozygous mutant TT, 35% CC and 22.5% CT, compared to 39.4% CC, 51.5% CT and 9.1% TT among healthy controls. This polymorphism was positively correlated to ACS risk of development in the CT genotype and the additive transmission model (OR [95% CI]=0.49 [0.16-0.99], P=0.002; OR [95% CI]=2.17 [0.94-2.72], P=0.02), increasing-thus- the association of this polymorphism with the risk of pathology occurrence.

Conclusion: To the best of our knowledge, our study is the first in Morocco to assess the effect of the C3435T MDR1 polymorphism on Clopidogrel response in a sample of Moroccan ACS patients; we also tried to explore the frequency of this polymorphism among Moroccan ACS patients and compare them to healthy individuals. The distribution of the mutant allele in Clopidogrel resistance groups, among ACS sub-types, and also in cases compared to controls and there correlations, suggest a potential association of this variant with Clopidogrel resistance and ACS occurrence risk in our population. Understanding the functional and clinical consequences of this *MDR1* variant and others may provide a basis for treating patients more effectively. If this variability in response could be assigned to a mutation in the *MDR1* gene, patients could be screened and appropriate dose adjustments could be made on the basis of their *MDR1* genotype.

Keywords: ACS; Clopidogrel resistance; C3435T MDR1 polymorphism; Moroccan population.

Introduction

Clopidogrel is an oral thienopyridine prodrug, usually prescribed for ACS patients as dual anti-platelet therapy in combination with aspirin. The efficacy of its metabolite active to decrease platelet aggregation by selectively and irreversibly blocking the P2Y12 receptor located on the surface of platelets, make of it the therapy of choice [1]. Once the P2Y12 receptor is inactivated, the association G-protein can no more inhibit adenylyl cyclase, inducing –thus- increased cAMP, and inhibiting the phosphoinositide 3-kinase (PI3K), as a result, the expression of glycoprotein IIb/IIIa subsequently decreases [2].

In the normal case, the glycoprotein IIb/IIIa mediates fibrinogen binding, leading to platelet aggregation and formation of a blood clot (Thrombus). This is what makes the prevention of this pathway by Clopidogrel and other ADP receptors inhibitors very essential in the treatment of thromboembolic events by decreasing platelet aggregation [1].

An inter-individual variability of response to Clopidogrel has been widely described; not all patients reach the same degree of benefit from

the given drug, they also still experience thrombotic events even after receiving the treatment [3]. The most part of this heterogeneity in response remains unclear, while some clinical factors that we know may explain just a small fraction of it (Smoking, use of PPIs (Proton Pump Inhibitors), lipophilic statins, calcium channel blockers, high pretreatment platelet reactivity…) [4,5-7]. Genetics may also explain another fraction of this variability in response to Clopidogrel [8,9].

ABCB1 gene

The physiology of drug transport is a key determinant of drug absorption, bioavailability, renal and biliary excretion, and its penetration into brain and other tissues. These specific functions may be modulated by several factors, and importantly by genomic variation [10]. Human ATP-binding cassette (ABC) transporters, especially ABCB1 (ATP-binding cassette, sub-family B, member 1), have been for a long time ago the focus of studies trying to access their effect on mediating drug resistance in a multitude of human pathologies, such as ACS [11]. This protein ensures the absorption of the pro-drug of Clopidogrel from the gastro-intestinal tract into the bloodstream, and transports it back into the intestinal lumen inhibiting thus the absorption [12,13]. Human multidrug resistance 1 (*MDR1*) gene, also called ABCB1, is located on chromosome 7q21.1, and is composed of 28 exons [14,15]. The product of this gene is a protein of 170 KD (1280 amino acids), called P-gp (P-glycoprotein), an energy-dependent efflux pump, involved in extrusion of a big variety of toxins as well as dietary and environmental carcinogens, and drugs [16-20].

Its activity consists of pumping out of cells, numerous compounds, such as drugs, using the energy derived from ATP hydrolysis. This activity has important pharmacokinetic and pharmacodynamic consequences [10]. The P-gp is expressed in several human tissues (gut, liver, kidney) [21]. In the apical membranes of intestinal, renal and hepatic epithelial cells, it acts on absorption and elimination of its substrates; within the apical membranes of capillary endothelial cells of the brain, it acts by limiting the penetration of drugs to the CNS [10]. The *MDR1* gene is highly polymorphic and more than 100 polymorphic sites with a minor allele frequency higher than 5% were discovered [22]. One of the most widely described as having an association with intestinal P-gp expression and activity *in vivo* is the C3435T (rs 1045642) [23]. Localized in exon 26, this polymorphism consists of a C-to-T exchange at cDNA 3435 position of the *MDR1* gene; it has no consequences on the amino acid sequence of the P-gp, but affects its function [23].

This synonymous variant of the *MDR1* gene alters gene expression, protein activity and stability of its RNAm, and substrate concentration and specificity [24-26]. It results in over-expression of the P-gp, associated to decrease Clopidogrel active metabolite, increased on-treatment platelet reactivity, and increased cardiovascular events [27-29]. Thus, carriers of the homozygous mutant genotype TT have lower intestinal P-gp expression, compared to carriers of the wild type genotypic profile CC [30,31]. The mechanism whereby such synonymous SNP exerts this effect still remain uncertain; it may be a marker for a functional SNP or conceivably be functional; it may also alter translation process [24]. Several studies have explored the association of this polymorphism with ACS risk, among different populations, but have found inconsistent and confusing results. The objectives of our study are, first, to determine the frequency of the C3435T *MDR1* polymorphism among Moroccan ACS and healthy subjects; and second, to assess its effect on Clopidogrel response in a sample of Moroccan ASC patients.

Materials and methods

Study population

Recruited patients were those having documented antiplatelet therapy (Clopidogrel), a VerifyNow P2Y12 platelet function test, and no more heparin in their blood. Excluded ones are those having incomplete clinical data or no platelet function test results. A baseline P2Y12 platelet function test was performed to all patients in this study, to evaluate their platelet function state. Blood samples were collected from 40 unrelated ACS Moroccan patients and compared to 99 apparently healthy subjects whose C3435T *MDR1* genetic results were previously reported in the study of Kassogue et al. [31], and showing no symptoms of coronary artery diseases. Clinical data concerning risk factors, biological parameters and the Verify-Now test results were collected; an informed consent, approved by the Ethical Committee of the University of Hassan II, School of Medecine, Casablanca, was signed by each patient and control before entering the study. The study protocol we followed was previously described in our paper in press [32,33].

DNA Extraction

Venous blood from all patients was collected in EDTA tubes. Samples were manipulated directly or stored at -20°C until extraction of DNA. Genomic DNA was extracted from blood leukocytes using the standard method of salting out [34-37].

Genotype Determination

We used PCR-RFLP to genotype samples for the C3435T MDR1 polymorphism, as previously described by Tanabe et al. [38]. Genotyping of this variant was performed by amplification from 50 to 100 ng of genomic DNA, followed by digestion using 10 units of MboI restriction enzyme.

The digestion gave rise to three profiles: homozygous wild type CC (Two fragments of 130 and 76 pb), heterozygous TC (three fragments of 206, 130 and 76 bp), and homozygous mutated TT (one fragment of 206 bp). The digested product was separated on 3% agarose gel electrophoresis stained with 0.5 µg/mL ethidium bromide (BET), and visualized with UV rayons.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 software. Chi square test ($\chi 2$) was used to determine statistical significance of association/non-association between genotypes and classical risk factors. Hardy–Weinberg Equilibrium test (HWE) was performed for cases and controls groups. Odds ratio (OR) were calculated to estimate the association between genotypes and ACS risk, using a Confidence Interval (CI) of 95%. Significance was approved at P value less than 0.05.

Results

Characteristics of the study population

The distribution of C3435T-MDR1 polymorphism was in HardyeWeinberg equilibrium (HWE) for controls group but not for cases one (Table 1). The average age was 57.23 ± 8.49 for patients *vs.* 31.78 ± 12.94 for healthy controls. There was a predominance of male

among cases (52.5%) and female among controls (68%) (Table 2). (Table 3) describes the routine pathology data for our SCA patients; 56.75% of these patients were under IPP, when 43.25% were not.

Verify now results *vs.* Risk factors

Patients were placed into resistant and non-resistant groups, based on their platelet function test results, and the baseline characteristics of these patients correlated to resistance groups are shown in (Table 4): HTA, Smoking, Creatinine and Sex were the risk factors that showed statistically significant association with Clopidogrel resistance (P=0.05; P=0.05; P=0.05 and P=0.04 respectively).

Allelic Frequencies

When correlating the C3435T *MDR1* genotypes to the classical risk factors of the pathology, only one statistically significant association was found with personal antecedent, among SCA patients; no association was detected with the other risk factors.

	EHW Cases		**EHW Controls**	
	X^2 square	P-value>0.05	X^2 square	P-value>0.05
C3435T MDR1	6	<0.05	1.3	0.25*

Table 1: Hardey-Weinberg Equilibrium (HWE) for cases and controls groups.

	SCA patients (N=40)	**Controls (N=100)**
Age (years)	57.23 ± 8.49	31.78 ± 12.94
Age of disease occurrence (years)	54.16 ± 8.31	
Sex		
Male	21 (52.5%)	32 (32%)
Female	19 (47.5%)	68 (68%)
Ethnicity		
Arab	32 (80%)	71 (71%)
Berber	8 (20%)	29 (29%)

Table 2: Description of ACS study population.

Parameters	**SCA patients**
Total Cholesterol (g/l)	1.91 ± 0.67
HDL (g/l)	1.82 ± 6.58
LDL (g/l)	1.34 ± 0.77
Triglycerides TG (g/l)	1.39 ± 0.56
Glucose (g/l)	1.47 ± 0.89
Creatinine (mg/l)	11.9 ± 10.87
Fibrinogene	3.72 ± 1.2
HB (g/dl)	14.15 ± 3.26
GB (elts/mm^3)	10397.78 ± 14972.03
Pq (elts/mm^3)	235934.57 ± 114017.04
BMI (kg/m^2)	26.86 ± 3.7
IPP	
(+)	21 (56.75%)

(-)	16 (43.25%)

Table 3: Routine pathology data of our ACS patients.

	Non Resistants %	**Resistants %**	**P-value**
Age	57 ± 8.48	59.25 ± 8.01	0.4
Sex			0.04*
Male	21 (58.33%)	0 (0%)	
Female	15 (41.67%)	4 (100%)	
SCA TYPE			0.3
ST (+)	9 (25%)	2 (50%)	
ST (-)	27 (75%)	2 (50%)	
familial ACD			NC
presence	0 (0%)	0 (0%)	
abscence	36 (100%)	4 (100%)	
personal ACD			0.5
presence	21 (58.3%)	2 (50%)	
abscence	15 (41.7%)	2 (50%)	
Diabete			0.6
presence	18 (50%)	2 (50%)	
abscence	18 (50%)	2 (50%)	
HTA			0.05*
presence	19 (52.78%)	4 (100%)	
abscence	17 (47.22%)	0 (0%)	
Dyslipidemia			0.7
presence	10 (27.77%)	2 (50%)	
abscence	26 (72.23%)	2 (50%)	
Smoking			0.05*
presence	17 (47.22%)	0 (0%)	
abscence	19 (52.78%)	4 (100%)	
Creatinine (mg/l)	11.41 ± 10.87	19 ± 4.34	0.05*
Fibrinogene	3.59 ± 1.2	5.88 ± 1.21	0.3
Pq	245937.5 ± 194017.04	129236.7 ± 84770.54	0.5
IPP			0.5
used	19 (52.78%)	2 (50%)	
non used	17 (47.22%)	2 (50%)	

Table 4: Baseline characteristics of our SCA patients *vs.* Verify now test results.

Risk factor		SCA patients		P-value<0.05
	N %	Hz %	muté %	
Familial ACD				NC
presence	0	0	0	
absence	14 (35%)	9 (22.5%)	17 (42.5%)	
personal ACD				0.02*
presence	9 (39.1%)	8 (34.8%)	6 (26.1%)	
absence	5 (29.4%)	1 (5.9%)	11 (64.7%)	
HTA				0.2
presence	9 (39.1%)	3 (13.04%)	11 (47.8%)	
absence	5 (29.4%)	6 (35.3%)	6 (35.3%)	
Smoking				0.2
presence	5 (29.4%)	6 (35.3%)	6 (35.3%)	
absence	9 (39.16%)	3 (13.04%)	11 (47.8%)	
Diabetes				0.6
presence	6 (30%)	4 (20%)	10 (50%)	
absence	8 (40%)	5 (25%)	7 (35%)	
Dyslipidaemia				0.3
presence	3 (30%)	1 (10%)	6 (60%)	
absence	11 (36.7%)	8 (26.7%)	11 (36.7%)	

Table 5: C3435T M*DR1* genotypes distribution *vs.* risk factors.

Table 6 shows the distribution of patients ACS type (ST (+) and ST (-)) according to the C3435T *MDR1* genotypic profiles: The majority of ST (+) patients were carrying the mutant allele (63.64%) in its heterozygous and mutant forms (54.5% and 36.4%), compared to a small fraction of them (9.1%) that were carrying the wild type allele in its homozygous form. For ST (-) group, equal distribution of the wild type and mutant alleles was found (50% for each one), with 44.8% that were CC or TT, and only 10.3% that were CT.

		SCA patients				**P-value<0.05**
	N %	Hz %	mutant %	wild type allele	mutant allele	0.007*
ST (+)	1 (9.1%)	6 (54.5%)	4 (36.4%)	36.36%	63.64%	
ST (-)	13 (44.8%)	3 (10.3%)	13 (44.8%)	50%	50%	

Table 6: C3435T *MDR1* genotypes distribution *vs.* ACS sub-groups.

Distribution of resistant and non-resistant patients according to the C3435T *MDR1* genotypes is reported in (Table 7): The most part of the resistant group of patients were carrying the mutant allele (62.5%), 50% were carrying the homozygous mutant genotypic profile, 25% were heterozygous and 25% homozygous wild type. For the non-resistant patients, 41% were homozygous mutant, 22.2% heterozygous and 36.1 % homozygous wild type.

Allelic and genotypic frequencies among cases and controls are reported in (Table 8): 42.5% of the cases were carrying the homozygous mutant genotype TT, when only 9.1% were TT in the controls group. Wild type and heterozygous frequencies were 35% and 22.2% among cases *vs.* 39.4% and 51.5% among controls respectively. A statistically significant association was found with the heterozygous genotype TC (OR [95% CI]=0.49 [0.16-0.99], P=0.002). There was also a positive correlation with the additive transmission model, but not the dominant and recessive models (OR [95% CI], P=2.17 [0.94-2.72], P=0.02), increasing-thus- the association of this polymorphism with the risk of pathology occurrence.

		SCA patients				**P-value<0.05**
	N %	Hz %	mutant %	wild type allele	mutant allele	0.5
Non-Resistant	13 (36.1%)	8 (22.2%)	15 (41.7%)	47.20%	52.80%	
Resistant	1 (25%)	1 (25%)	2 (50%)	37.50%	62.50%	

Table 7: C3435T *MDR1* genotypes distribution *vs* verify now test results.

	Genotypes/alleles	**Cases (%)**	**Controls (%)**	**OR (95% CI)**	**P-value**
C3435T *MDR1*	**CC**	14 (35%)	39 (39.4%)	1	
	CT	9 (22.5%)	51 (51.5%)	0.49 [0.16-0.99]	0.002*
	TT	17 (42.5%)	9 (9.1%)	5.26 [1.28-9.87]	0.5
	CC+CT (b)	23 (57.5%)	90 (90.9%)	1	
	TT	17 (42.5%)	9 (9.1%)	7.39 [2.09-13.74]	0.5
	CC (c)	14 (35%)	39 (39.4%)	1	
	CT+TT	26 (65%)	60 (61.6%)	1.21 [0.42-1.85]	0.5
	C (d)	37 (46.25%)	129 (65.85%)	1	
	T	43 (53.75%)	69 (34.15%)	2.17 [0.94-2.72]	0.002*

Table 8: Allelic and genotypic frequencies of C3435T MDR1 polymorphism among cases and controls.

Discussion

Acute Coronary Syndrome (ACS) in one of the most associated pathologies to a risk of mortality and morbidity, in all its forms; which makes of it a big challenge to clinicians. The treatment of ACS typically includes antithrombotic and antiplatelet agents, in addition to percutaneous coronary or surgical interventions [38,39]. Clopidogrel is currently used in routine combined to aspirin as dual antiplatelet therapy, and has shown important efficacy [40-44]. Despite all its benefits, a significant inter-individual variability in response to Clopidogrel has been widely described. As consequences to this variability in response to treatment, some patients show non-responsiveness or decreased inhibition of platelet aggregation after being treated by Clopidogrel, which has been associated with increased risk of cardiovascular events [45,46]. One of the first causes of this variability lies in the pharmacokinetics of Clopidogrel. This prodrug needs to be –first- transported by the P-gp protein (*ABCB1* gene product), and metabolized –after- by the Cyp 450 system in the liver; the active metabolite then irreversibly inhibits the *P2Y12*, the direct receptor of Clopidogrel [47]. Genetic variations in these genes (*ABCB1, CYP3A4, CYP2B6, P2Y12*) have been associated with this inter-individual variability in response to Clopidogrel, and may – thus-explain an important fraction of it [39,48].

Interestingly, Multi-Drug Resistance gene-1 (MDR-1) polymorphism has been found to influence oral bioavailability of clopidogrel and prognosis of ACS patients. Taking these backgrounds into account, the purpose of the present study – as being the first to do it in Morocco- was to determine the frequency of C3435T MDR1 polymorphism among Moroccan ACS patients; and to evaluate the correlation between Clopidogrel resistance and genetic testing represented by the C3435T MDR1 polymorphism, in a sample of Moroccan ACS patients.

Our study population was for the most part of it composed of male (52.5%), when a predominance of female was noted among healthy controls (68%) (Table 2). Similar distribution was reported in the study of Ayaz et al. [48], investigating the association of MDR1 C3435T and G2677T/A polymorphisms with plasma platelet-activating factor levels and coronary artery disease risk in Turkish population. The average age was 57.23 ± 8.49 and 31.78 ± 12.94 in cases and controls groups respectively.

56.75% of our patients were under PPIs *vs.* 43.25% that were not (Table 3). Proton-pump inhibitors (PPI) are known to potentially affect the Clopidogrel platelet inhibition relationship [49,50]. Several studies have reported an inhibitory effect of PPIs (especially omeprazole) on the antiplatelet efficacy of Clopidogrel [51-55]; when others did not report any interaction between PPIs and Clopidogrel [56-58]. In 2010, The Committee on Human Medicinal Products (CHMP) has reported that the concomitant use of clopidogrel and omeprazole or esomeprazole in clinical practice was discouraged, and that, in patients under PPIs, use of pantoprazole in place of omeprazole or lansoprazole is recommended; this come from the fact that pantoprazole does not appear to have the significant inhibitory effect on the cytochrome enzyme CYP2C19 that other PPIs such us omeprazole have [59].

Our patients were placed into resistants and non-resistants, according to their platelet function test results, and were then correlated to the baseline characteristics of the studied population (Table 4). HTA, Smoking, Creatinine and Sex were the risk factors that showed statistically significant association with Clopidogrel resistance (P=0.05; P=0.05; P=0.05 and P=0.04 respectively). 58.33% of the non-resistant patients were male, when the whole resistant group was

female (Table 4). Jun-Feng et al. have found similar results concerning the predominance of female among resistant and male among non-resistant patients, and the positive correlation of HTA and Creatinine with Clopidogrel resistance, but not for the rest of risk factors [60]. Other studies report a as well a significant association of smoking with clopidogrel resistance [61,62]. Several studies have suggested that women do not accrue equal therapeutic benefit of antithrombotic therapy [63,64]. Although multiple contributing factors have been described (Differences in vessel wall biology between men and women; the direct influence of sex hormones (oestrogens, progesterone or androgens) on platelets and their indirect effect on the vasculature), the physiological mechanism behind this gender disparity remains unclear [65].

A statistical comparison was held between distribution of C3435T MDR1 polymorphism among ACS patients and traditional risk factors; we found a statistically significant association with personal antecedent (P=0.02); no other significant association was found with the other risk factors (Table 5). Correlation between this polymorphism and ACS sub-groups (ST+ and ST-) showed that the majority of ST(+) patients were carrying the mutant allele (63.64%) in its heterozygous and mutant forms (54.5% CT and 36.4% TT), when only a small fraction of them was carrying the wild type allele in its homozygous form (9.1% CC). ST (-) group showed equal distribution of the wild type and mutant alleles (50% for each one), with 44.8% of ST (-) patients having the CC or TT genotypes, and only 10.3% that were CT. This distribution showed statistically signification association of the C3435T polymorphism with the ACS sub-types (P=0.007) (Table 6).

Distribution of resistant and non-resistant patients according to the C3435T *MDR1* genotypes showed that the majority of the resistant group were carrying the mutant allele (62.5%), 50% were carrying the homozygous mutant form TT, 25% were heterozygous CT and 25% homozygous wild type CC. For the non-resistant group, 41% were homozygous mutant TT, 22.2% heterozygous CT and 36.1 % homozygous wild type CC (Table 7). Allelic and genotypic frequencies among cases and controls reported in (Table 8) show that: 42.5% of the cases were carrying the homozygous mutant genotype TT, when only 9.1% were TT in the controls group. A statistically significant association was found with the heterozygous genotype TC (OR [95%CI], P=0.49 [0.16-0.99], P=0.002). There was also a positive correlation with the additive transmission model, but not the dominant and recessive ones (OR [95%CI], P=2.17 [0.94-2.72], P=0.02), increasing-thus- the association of this polymorphism with the risk of pathology occurrence. The study performed by Jaitner et al. trying to assess the association of ABCB1 C3435T genotypes with the antiplatelet efficacy of clopidogrel and the risk of stent thrombosis (ST) in large cohorts of clopidogrel-treated patients undergoing percutaneous coronary intervention, reported that the C3435T MDR1 polymorphism did not influence the risk of ST or the response to Clopidogrel in Clopidogrel treated patients [66]. Association between C3435T MDR1 and Clopidogrel resistance is not as well established, and studies have reported inconsistent and conflicting results. Śpiewak et al. have found a significant correlation between this polymorphism and Clopidogrel resistance and the availability of its active metabolite [67]. This may be explained by the decreased intestinal absorption of Clopidogrel in patients carrying the TT mutant genotype, compared to those carrying the CC wild type one. Several other studies reported similar findings, when others reported contracting results. Another study performed by Wallentin et al. has found that the ex-vivo addition of the active metabolite of Clopidogrel can overcome its resistance, and induce higher platelet inhibition regardless of the initial platelet reactivity after 600 mg loading dose and 75 mg maintenance dose of Clopidogrel. This suggests that variability in antiplatelet response to clopidogrel depends more on plasma levels of its active metabolite (that can be affected by *ABCB1* and Cytochrome P450 genetic variations) than on the platelet receptor properties [67,68].

Conclusion

As causing no change on the protein sequence and being located in a non-regulatory region of the *MDR1* gene, it may be conceivable that the C3435T polymorphism is not causative for differences in P-gp expression. It may be linked to other changes in regions that control expression of the *MDR1* gene (the promoter/enhancer region) or regions that are relevant for mRNA processing. Nevertheless, this SNP appears to allow the differentiation of alleles with distinct MDR-1 expression and activity. Therefore, understanding the functional and clinical consequences of this MDR1 variant and others may provide a basis for treating patients more effectively. If this variability in response could be assigned to a mutation in the *MDR1* gene, patients could be screened and appropriate dose adjustments could be made on the basis of their *MDR1* genotype. Since we found significant associations between some patient characteristics and Clopidogrel resistance, these characteristics should be considered prior to the administration of Clopidogrel. This would help distinguish patients that are likely to respond to the drug from those that would likely be resistant. While this study is promising, it is partly limited by the small sample size, and should be followed up by multi-center clinical trials with a greater number of patients in order to confirm and verify our findings.

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