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Genetic polymorphisms in *ADRB1*, *ADRB2* and *CYP2D6* genes and response to beta-blockers in patients with acute coronary syndrome

Celia Castaño-Amores [a], Alba Antúnez-Rodríguez [b], Ana Pozo-Agundo [b], Sonia García-Rodríguez [b], Luis Javier Martínez-González [c], Cristina Lucía Dávila-Fajardo [d,*]

[a] *Pharmacy Department, Hospital Universitario Santa Bárbara, Puertollano, Spain*
[b] *GENYO, Genomics Unit, Centre for Genomics and Oncological Research: Pfizer, University of Granada, Andalusian Regional Government (GENYO), Granada, Spain*
[c] *University of Granada, Department of Biochemistry and Molecular Biology III and Immunology, Faculty of Medicine, PTS, Granada, Spain*
[d] *Pharmacy Department, Instituto de Investigación Biosanitaria de Granada (ibs.Granada), Hospital Universitario Virgen de las Nieves, Granada, Spain*

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ABSTRACT

Betablockers (BBs) are prescribed for ischaemia in patients with acute coronary syndrome (ACS). In Spain, bisoprolol and carvedilol are the most prescribed BBs, but patients often had to discontinue them due to adverse effects. Single nucleotide polymorphisms (SNPs) in *ADRB1*, *ADRB2* and *CYP2D6* genes have strong evidence of pharmacogenetic association with BBs in heart failure or hypertension, but the evidence in ACS is limited. Therefore, our study focuses on investigating how these genes influence the response to BBs in ACS patients. We analysed the association between SNPs in *ADRB1* Gly389Arg (rs1801253) and Ser49Gly (rs1801252), *ADRB2* Gly16Arg (rs1042713) and Glu27Gln (rs1042714), and *CYP2D* 6 (*2– rs1080985*, **4- rs3892097*, **10* – rs1065852) and the occurrence of bradycardia/hypotension events during one year of follow-up. We performed an observational study and included 285 ACS-PCI-stent patients. A first analysis including patients treated with bisoprolol and a second analysis including patients treated with other BBs were performed. We found that the presence of the *G* allele (Glu) of the *ADRB2* gene (rs1042714; Glu27Gln) conferred a protective effect against hypotension-induced by BBs; OR (CI 95%) = 0,14 (0,03−0,60), $p < 0.01$. The *ADRB2* (rs1042713; Gly16Arg) *GG* genotype could also prevent hypotensive events; OR (CI 95%) = 0.49 (0.28–0.88), p = 0015. SNPs in *ADRB1* and *CYP2D6 * 2*, *CYP2D6 * 4* werent associated with primary events. The effect of *CYP2D6 * 10* does not seem to be relevant for the response to BBs. According to our findings, SNPs in *ADRB2* (rs1042713, rs1042714) could potentially affect the response and tolerance to BBs in ACS-patients. Further studies are necessary to clarify the impact of *ADRB2* polymorphisms.

1. Introduction

Beta-blockers (BBs) are prescribed for ischaemia secondary to acute coronary syndrome (ACS). They are initially prescribed at low doses and gradually titrated to avoid adverse effects (AEs) such as bradycardia or severe hypotension [1,2]. These drugs are blockers of the beta-adrenergic receptors and work by reducing the heart rate (HR), contractility and blood pressure (BP). Chronic BB treatment is recommended by cardiology guidelines, particularly in patients with impaired left ventricular function or residual coronary involvement with angina, unless contraindicated [1–4]. However, the occurrence of AEs and patient intolerance often lead to discontinuation of BBs, with approximately 25% of patients eventually discontinuing their medication [5,6].

In Spain, according to the Spanish TRECE (Treatment of Coronary Disease in Spain) registry, the most commonly prescribed BB was atenolol (43.9%), followed by bisoprolol (30.9%), carvedilol (22.1%), metoprolol (2.3%) and others (0.8%). Patients receiving atenolol, bisoprolol or metoprolol had a higher prevalence of Resting Heart Rate (RHR) < 70 beats per minute (bpm) [7]. In Caucasians, variability in BBs response and BP change has been reported, which may be explained by genetic and non-genetic causes [8].

Genes encoding metabolic enzymes are the most studied due to their clinical relevance, as well as genes encoding biological drug targets. The *CYP2D6, ADRB1 and ADRB2* genes have robust evidence for pharmacogenetic (PGx) association with BBs, reaching level 1 of evidence as Very Important Pharmacogenes (VIPs) according to PharmGKB database

* Corresponding author.
E-mail address: cristinal.davila.sspa@juntadeandalucia.es (C.L. Dávila-Fajardo).

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[9]. Carvedilol and metoprolol have been extensively investigated in PGx studies [10]. The PGx evidence for bisoprolol has been summarized in a systematic review with meta-analysis conducted by our group [11].

1.1. CYP2D6

Bisoprolol is metabolised by CYP3A4 and CYP2D6 isoenzymes and the remainder is excreted unchanged by the kidneys [5]. Other BBs such as carvedilol, metoprolol or propranolol are mainly metabolised by *CYP2D6* [10].

Poor metabolizers (PM) and intermediate metabolizers (IM) of metoprolol convert less of the drug to its inactive form. As a result, higher drug concentrations may lead to increased rates of bradycardia [12–14]. The Dutch Pharmacogenetics Working Group (DPWG) recommends stepwise titration of metoprolol [10,11,15,16]. PGx associations with HR appear robust, while data for BP suggest no significant association [10,17,18]. IM/PM of carvedilol, eliminate the drug less efficiently than normal metabolisers. However, the FDA and DPWG have not made specific dosing recommendations [10,19,20]. Several studies have been conducted on bisoprolol, but results regarding the major *CYP2D6* alleles are inconclusive and show conflicting results in different populations. [21,22]. Some authors have described variations in bisoprolol requirements in patients with ACS based on their *CYP2D6 * 10* and *CYP2D6 * 4* genotypes [11,80].

1.2. Beta-adrenergic receptors (ADRB)

The beta-adrenergic receptor beta-1 (*ADRB1) and* beta-2 (*ADRB2)* genes encode beta-1 and beta-2 receptors. These are G-protein-associated receptors found mainly in heart tissue [10]. The most studied polymorphisms of these genes are single nucleotide polymorphisms (SNPs); rs1801252 (*ADRB1;* Ser49Gly), rs1801253 (*ADRB1;* Gly389Arg), rs1042713 (*ADRB2;* Gly16Arg) and rs1042714 (*ADRB2;* Glu27Gln) [10,11].

Numerous studies have investigated the association between these variants and HR or BP response to BBs. However, most of them did not find PGx associations [11]. The studies with positive results mainly focused on *ADRB1* with BP variations [10,24]. For example, *Lui J et al.* described that individuals carrying the Arg389Arg polymorphism (rs1801253) had lower BP values after metoprolol administration compared to Gly389Gly carriers [24]. Other studies are consistent with these findings. [24–26]. T*erra SG et al.* [27] described that HF Gly389 (rs1801253) patient carriers needed other treatments to control HF due to decompensation. However, some authors found no HR differences with SNPs in *ADRB1* with metoprolol, carvedilol, or bisoprolol. [23, 28–31].

The majority of studies investigating *ADRB2* polymorphisms with BBs did not find PGx interactions [6,28], but other authors described associations for HR and BP changes. *Sain MH et al.* [32] reported that Glu27Gln (rs1042714) and Gly16Arg (rs1042713) were associated with the degree of HR reduction. The reduction in HR with atenolol was greater with the Gln27 allele (rs1042714), $p = 0.01$. In contrast, *Kaye DM et al.* [33] reported that homozygous Gln27Gln patients responded poorly to carvedilol, as their LVEF did not improve as much as that of Glu27 carriers. *Sehrt D et al.* described a greater decrease in resting BP with carvedilol in carriers of the Gln27 variant [34]. For the Gly16Arg (rs1042713) polymorphism, a meta-analysis showed a better response to BBs in Arg16 carriers [32].

In a prospective study of more than 700 ACS patients treated with BBs, homozygous Gln27Gln patients had a higher mortality rate within 3 years follow-up (16%) than heterozygous Glu27Gln (11%) or homozygous Glu27Glu (6%) patients, $p = 0.03$. In addition, patients who were also homozygous for Arg16Arg and Gln27Gln had a higher risk of death than patients with the other diplotypes [35].

Several authors reported specific results for bisoprolol [11]. Most studies investigated these SNPs in patients with HF or hypertension. *Lee et al.* [36] observed that Arg389Arg (*ADRB1*) patients required higher doses of bisoprolol compared to Gly carriers (Gly389Arg or Gly389Gly), but without significant changes in HR. *Rau et al.* [21] also found no association for Gly389Arg and Ser49Gly (*ADRB1*) polymorphisms. Similarly, *de Groote et al.* [28], did not obtain results for the SNPs of the *ADRB*1 and *ADRB2* genes. In contrast, some authors reported positive results with potential PGx interactions for these polymorphisms [11,30, 37,38].

The available evidence from studies on drug-gene interactions with bisoprolol is rather limited and is classified as level 3 evidence (low evidence) [39]. Therefore, it may be valuable to investigate the influence of polymorphisms in *ADRB1* and *ADRB2*, and *CYP2D6* on the response to bisoprolol and other BBs in patients with ACS who have undergone percutaneous coronary intervention (PCI) with stent.

2. Material and methods

2.1. Patients and treatment

In this study we evaluated the cohort of patients thoroughly described in detail by *Dávila-Fajardo CL et al.* [40,41]. Briefly we performed a non-randomized experimental study in 719 ACS patients recruited at the San Cecilio University Hospital, Granada, Spain. The prospective *CYP2C19/ABCB1* genotype-guided antiplatelet strategy (intervention group, $n = 317$) was compared with a retrospective non-guided strategy (control group, $n = 402$). Follow-up was 12 months. Recruitment took place from April 2010 to September 2013. The study protocol was approved by the Granada Research Ethics Committee. In the study presented in this article, all patients belonged to the prospective *CYP2C19/ABCB1* genotype-guided antiplatelet group, receiving only the antiplatelet agent according to the pharmacogenetic test and prescribing BBs based on clinical practice without pharmacogenetic testing. We selected 285 patients from that cohort whose saliva sample was available for genetic analysis and whose digital medical records were accessible for clinical data collection.

2.2. Clinical evaluation

The primary objective of this study was to evaluate the association of the *ADRB1* Gly389Arg (rs1801253), *ADRB1* Ser49Gly (rs1801252), *ADRB2* Gly16Arg (rs1042713), *ADRB2* Glu27Gln (rs1042714), *CYP2D6 * 2* (rs1080985), *CYP2D6 * 4* (rs3892097), *CYP2D6 * 10* (rs1065852) polymorphisms with the occurrence of primary events (bradycardia and hypotension) during the treatment with BBs and up to 12 months after baseline (BL), considering BL the first day of treatment with BB from hospital admission. Patients treated with more than one BB were included once in the overall analysis and then separately in the groups of receiving bisoprolol or other BBs.

Events occurring during the 1-year follow-up period were recorded by the investigators using hospital medical records, pharmacy records, and patient questionnaires. The primary endpoint "bradycardia" was defined as a HR below 60 beats per minute (BPM). Hypotension was defined as a systolic pressure level below 90 mmHg and/or a diastolic pressure levels below 60 mmHg.

2.3. DNA extraction and genotyping

For genotyping, 4 saliva samples were collected from each recruited patient using sterile cotton swabs. DNA was extracted using modification of the salting-out extraction method developed by *Freeman et al.* [42], as described by *Gomez-Martín A. et al.* [43]. SNPs were genotyped using allele-specific hybridisation probes, KASP (Kompetitive Allele-Specific PCR) from LGC Biosearch™ Technologies (Teddington, Middlesex, UK) and analysed using the KlusterCaller software (LGC Genomics, Hoddesdon, Hertfordshire, UK) [44]. The call rate for all SNPs tested was > 98%. Quality control of the genotyping results was

performed with negative controls and randomly selected samples included as duplicates.

2.4. Statistical analysis

The statistical analysis was performed using the R commander (V.2.3.0) statistical package of the R statistics (V.3.6.2.) programme. First, a descriptive analysis of the clinical parameters was performed. We examined the Hardy-Weinberg (H-W) equilibrium of each SNP for the total number of patients. To check for possible confounding, the association between the covariates and the dependent variables (primary AE) and the independent variables (genetic variables) was analysed to determine the influence of the covariates on the response or their possible association with genetic polymorphisms. The $\chi 2$ (Chi-square) test was used to compare proportions for qualitative variables. If the frequency was < 5 in more than 20% of the cells of each of the contingency tables constructed, Fisher's exact test was used. The analysis of variance (ANOVA) or the Kruskall-Wallis test was used to compare the quantitative variables with the qualitative variables, after checking normality with the Shapiro-Wilks test and checking variances with the Barlett test. The variables that showed an association with the polymorphisms or the response variables in this bivariate analysis ($p < 0.1$) were included in the final multivariate model.

We then performed the association analysis between polymorphisms and the primary events (bradycardia/hypotension) in the whole population. We performed the same analysis for the subgroup of patients treated with bisoprolol. In addition, the association of polymorphisms with the response to BBs other than bisoprolol was studied as a secondary objective. For this purpose, a bivariate analysis was carried out using the $\chi 2$ test - chi-square. A multivariate logistic regression analysis was performed including variables previously shown to be associated in the bivariate analysis. The analysis was also adjusted for polymorphisms using the Bonferroni correction to control for type I error.

2.5. In silico analysis

An analysis of the studied variants in the *ADRB1* and *ADRB2* genes was performed using the Ensembl Variant Effect Predictor (VEP) tool to estimate the malignancy of the variants and the impact of the transcript changes (https://www.ensembl.org/info/docs/tools/vep/index.html) [45]. This tool includes the Sorting Intolerant From Tolerant (SIFT) score, which predicts whether an amino acid change is likely to result in a change in the functionality of the encoding protein. Variations with a score close to zero are more likely to be deleterious. If the score is less than 0.05 they are considered deleterious and if it is higher, they are considered tolerable. The Genotype-Tissue Expression (GTEx) tool was used to analyse the level of gene expression of the *ADRB1* and *ADRB2* genes in cardiac tissue and blood according to the SNPs studied. The data for the analyses described were obtained from the GTEx portal (https://gtexportal.org/home/faq#citePortal).

3. Results

3.1. Characteristics of patients

Baseline characteristics of the patients are shown in Table 1. Of the 285 patients recruited, 74 (25.9%) were women, the mean age was 63.5 ± 12 years, and almost all were Caucasian. Regarding CVD risk factors, 101 (35.4%) patients had diabetes, 174 (61%) had hypertension, 166 (58.2%) had dyslipidaemia, 109 (38.2%) were smokers, and 91 (31.9%) had a history of heart disease at the time of inclusion. Regarding CV history, 46 (16.1%) patients had experienced angina, 39 (13.6%) had a history of ACS, 15 (5.2%) had stroke, and 13 (4.5%) had HF. In addition, 24 (8.4%) patients had respiratory disease and 9 (3.1%) had renal impairment.

Regarding baseline CV treatment, 20 patients (7%) were taking acetylsalicylic acid (ASA), 140 (49%) antihypertensives, 108 (37.8%) antiplatelet agents, 2 (0.7%) anticoagulants, 115 (40.3%) lipid-lowering agents, and 72 (25.2%) patients were taking BBs; mainly bisoprolol (44.4%) and atenolol (29%).

Table 1
Baseline characteristics of patients.

Baseline characteristics: mean ± SD or n (%)	
Mean age (years)	63.5 ± 12 years
Sex female	74 (25.9%)
Caucasians	281 (98.6%)
Diabetes	74 (25.9%)
Hypertension	174 (61%)
Dyslipidemia	166 (58.2%)
Smokers	109 (38.2%)
CV history*	91 (31.9%)
Renal impairment	9 (3.1%)
Respiratory disease	24 (8.4%)
Patients previously treated with BBs	72 (25.2%)
Betablocker prescribed:	143 (50.1%)
-Bisoprolol	74 (26%)
-Carvedilol	49 (17.2%)
-Atenolol	19 (6.6%)
Nebivolol	
Hypotension	72 (25.3%)
Bradycardia	55 (19.3%)

* Cardiovascular (CV) history: angina/ACS/stroke/HF.

After hospital admission, all patients were prescribed an antiplatelet agent. In addition, 254 (89.1%) patients were prescribed an antihypertensive (ACEI or ARA-II), 57 (20%) diuretics, and 17 (6%) ivabradine. Statins for hypercholesterolaemia were prescribed to 267 (93.7%) patients. In addition, 255 (89.4%) patients were taking a proton pump inhibitor (PPI). Regarding the prescription of BBs, 143 (50.1%) patients were prescribed bisoprolol, 74 (26%) carvedilol, 49 (17.2%) atenolol, and 19 (6.6%) nebivolol. During the follow-up period, 3 patients treated with bisoprolol were also treated with other BBs, but they were included once in the total cohort in the group of patients treated with bisoprolol.

Hypotensive events occurred in 72 (25.3%) patients and bradycardia in 55 (19.3%) patients. Other AEs were recorded as secondary events. Dizziness was reported in 33 (11.5%) patients, syncope in 14 (5%) patients, BB dose reductions in 27 (9.5%) patients, and discontinuation due to AEs in 18 (6.3%) patients.

The genotype distribution of the studied SNPs is summarized in Supplementary Table S1. All genotypes and allelic frequencies of the variants studied were in H-W equilibrium ($p > 0.05$) when compared to the reference populations, except for the rs1801252 polymorphism (*ADRB1*; Ser49Gly).

3.2. Analysis of association between covariates and genetic polymorphisms and response

The rs1801252 (*ADRB*1; Ser49Gly) polymorphism showed an association with Angiotensin II-Receptor Antagonist (ARA-II) antihypertensive drugs ($p = 0.040$). The rs1801253 (*ADRB1*; Gly389Arg) polymorphism showed an association with age ($p = 0.081$), smoking ($p = 0.071$), dyslipidaemia ($p = 0.021$) and with ivabradine ($p = 0.025$). For the two selected polymorphisms of the *ADRB2* gene, no association was found with the covariates. Regarding *CYP2D6* polymorphisms, *CYP2D6 * 2* showed an association with the Caucasian ancestry ($p = 0.096$), dyslipidaemia ($p = 0.077$), statins ($p = 0.097$) and PPIs ($p = 0.086$). *CYP2D6 * 4* and *CYP2D6 * 10* polymorphisms showed no association with covariates.

In the analysis of the covariates with the primary events (bradycardia and hypotension), the following variables showed an association with bradycardia: female sex, dyslipidaemia, statins, ACEI, ARA-II, ivabradine, and PPIs. Associations were found for hypotension, dyslipidaemia, hypertension and ivabradine treatment. The results of these analyses are shown in detail in Supplementary Tables S2-S4.

3.3. Analysis of the association between polymorphisms and primary events

In this analysis, the response to BBs (bisoprolol/carvedilol/atenolol/nebivolol) in the whole population was compared with the variables bradycardia and hypotension according to the presence of the SNPs studied. The results are shown in detail in Tables 2 and 3.

3.3.1. Association analysis in the whole population with BBs

In this first bivariate analysis, the *CYP2D6 * 10* polymorphism showed an association with BBs set for the bradycardia event ($p = 0.046$). The association was maintained after covariate adjusted modelling ($p = 0.03$). A non-significant trend was observed for the *CYP2D6 * 4* polymorphism ($p = 0.07$). The results are shown in Table 2.

For the hypotension event, both polymorphisms in *ADRB2* showed significant association; rs1042713 (Gly16Arg) ($p = 0.015$) and rs1042714 (Glu27Gln) ($p < 0.01$). After adjustment for covariates, the association was maintained (Gly16Arg; $p = 0.027$), (Glu27Gln; $p < 0.01$). For the rs1042714 (Glu27Gln) polymorphism, the association was also maintained after adjustment for polymorphisms ($p = 0.01$). The results are shown in Table 3.

3.3.2. Association analysis with bisoprolol (Primary endpoint)

The association of polymorphisms with the primary events was analysed in patients treated with bisoprolol. For the bradycardia event, no association with SNPs was found in patients treated with bisoprolol (see Supplementary Table S5). For the hypotension event, the association previously observed for the rs1042714 (Glu27Gln) polymorphism in the whole population analysis was significant for bisoprolol ($p = 0.02$). The association persisted after adjustment for covariates ($p < 0.01$). The results are shown in detail in Table 4.

3.3.3. Association analysis with other BBs (Secondary endpoint)

As a secondary objective, the association of the polymorphisms with other BBs than bisoprolol was analysed. Because of the small number of patients treated with other BBs in each group, they were analysed together (atenolol/carvedilol/nebivolol).

For the bradycardia event, no association was found in this group of patients. However, for the hypotension event, the same association was found significant as above for the rs1042714 (Glu27Gln) polymorphism, $p < 0.01$. The association was maintained in models adjusted for covariates ($p = 0.02$) and for the other polymorphisms ($p = 0.03$) (Supplementary Tables S6 and S7).

3.4. 4 In silico analysis

To understand the effect of *ADRB* variants and gene expression in cardiac tissue and blood, in silico analyses were performed for the studied SNPs. Allelic changes of the four SNPs produce nonsense variants that result in amino acid changes in the sequences (Table 5). For the four SNPs, the changes in the protein seem to be tolerated ($p > 0.05$), although, for rs1042713 the SIFT score is close to the threshold of significance, with a p-value = 0.07. The GTEX analysis showed interesting results for the expression of the *ADRB2* SNPs. For rs1042713, the data show that the *GG* genotype has a higher expression in the aortic artery and in whole blood (Fig. 1). Similarly, for rs1042714 the *GG* genotype showed higher expression in whole blood.

For the SNPs of the *ADRB1* gene, it was observed that for rs1801252 there was a greater expression of the *AA* genotype in the coronary artery and in the atrial appendage of the heart, and for rs1801253 there was a greater expression of the *GG* genotype was also observed in these two tissues, as well as in the aorta. Table 6.

4. Discussion

BBs are widely used in several CV diseases. In the last decade, the influence of genetic polymorphisms on the response and tolerance to BBs has been studied, particularly in HF and hypertension [18,35,46,47]. *ADRB1* and *ADRB2* have been shown to have an effect on BP according to functional genotype, and the role of metabolising enzymes, such as CYP2D6, on the plasma level of BBs and their AEs (bradycardia or BP lowering), has also been extensively studied, although no consensus has yet been reached [48,49]. On the other hand, the currently available evidence on the influence of *ADRB1*, *ADRB2* and the role of *CYP2D6* in patients with ACS is rather limited [11].

In this study, we investigated whether the genetic variants of the *ADRB1, ADRB2,* and *CYP2D6* genes are associated with bradycardia and hypotension as major AEs in patients with ACS.

Table 2
Analysis of association between the SNPs in the global population (n = 285) treated with BBs with the event bradycardia.

		BRADYCARDIA		OR (CI 95%)	p-value	OR (CI 95%)[c]	p-value	OR (CI 95%)[d]	p-value
		YES (n = 55)	NO (n = 230)						
rs1801252 (*ADRB1*; Ser49Gly)[a]	A/A A/G - G/G	40 (72.7%) 15 (27.3%)	170 (73.9%) 60 (26.1%)	0.94 (0.49–1.83)	0.86	0.94 (0.47–1.91)	0.87	0.85 (0.43–1.70)	0.85
rs1801253 (*ADRB1*; Gly389Arg)[a]	C/C G/C - G/G	26 (47.3%) 29 (52.7%)	114 (49.6%) 116 (50.4%)	0.91 (0.51–1.64)	0.76	0.87 (0.45–1.66)	0.67	0.92 (0.50–1.68)	0.78
rs1042713 (*ADRB2*; Gly16Arg)[a]	G/G G/A - A/A	19 (34.5%) 36 (65.5%)	90 (39.1%) 140 (60.9%)	0.82 (0.44–1.52)	0.53	0.99 (0.51–1.92)	0.99	0.80 (0.38–1.68)	0.55
rs1042714 (*ADRB2*; Glu27Gln)[b]	G/C - C/C G/G	50 (90.9%) 5 (9.1%)	197 (85.7%) 33 (14.3%)	1.68 (0.62–4.51)	0.28	1.55 (0.54–4.44)	0.4	1.47 (0.47–4.60)	0.5
rs3892097 (*CYP2D6 *4*)[a]	C/C C/T - T/T	42 (76.4%) 13 (23.6%)	147 (63.9%) 83 (36.1%)	1.82 (0.93 −3.59)	0.07	1.86 (0.90–3.84)	0.08	0.70 (0.08–6.23)	0.74
rs1065852 (*CYP2D6 *10*)[a]	G/G G/A- A/A	41 (74.5%) 14 (25.4%)	139 (60.4%) 91 (39.6%)	1.92 (0.99–3.72)	**0.046**	2.13 (1.04–4.37)	**0.03**	2.49 (0.29–21.15)	0.35
rs1080985 (*CYP2D6 *2*)[a]	G/G C/G - C/C	29 (52.7%) 26 (47,3%)	131 (57%) 99 (43%)	0.84 (0.47–1.52)	0.57	1.08 (0.57–2.03)	0.82	1.01 (0.54–1.88)	0.99

[a] Dominant model;
[b] Recessive model;
[c] adjusted by covariates;
[d] adjusted by SNPs

Table 3
Analysis of association between the SNPs in the global population (n = 285) treated with BBs with the event hypotension.

		HYPOTENSION		OR (CI 95%)	p-value	OR (CI 95%)[c]	p-value	OR (CI 95%)[d]	p-value
		YES (n = 72)	NO (n = 213)						
rs1801252 (*ADRB1*; Ser49Gly)[a]	A/A	55 (76%)	155 (72.8%)	1.21	0.54	1.27	0.46	1.15 (0.60–2.20)	0.68
	A/G - G/G	17 (23.6%)	58 (27.2%)	(0.65–2.25)		(0.66–2.45)			
rs1801253 (*ADRB1*; Gly389Arg)[b]	C/C - G/C	67 (93.1%)	203 (95.3%)	0.66 (0.22–2)	0.47	0.63	0.47	0.83 (0.26–2.69)	0.76
	G/G	5 (6.9%)	10 (4.7%)			(0.19–2.13)			
rs1042713 (*ADRB2*; Gly16Arg)[a]	G/G	19 (26.4%)	90 (42.2%)	0.49	**0.015**	0.51	**0.027**	0.71 (0.36–1.41)	0.32
	G/A - A/A	53 (73.6%)	123 (57.8%)	(0.28–0.88)		(0.28–0.94)			
rs1042714 (*ADRB2*; Glu27Gln)[b]	G/G	2 (2,8%)	36 (16,9%)	0,14	**< 0.01**	0,12	**< 0.01**	0,17 (0,03–0,84)	**0.01**
	C/C - G/C	70 (97,2%)	177 (83,1%)	(0,03–0,60)		(0,02–5,32)			
rs3892097 (*CYP2D6 *4*)[a]	C/C	52 (72.2%)	137 (64.3%)	1.44	0.21	1.38	0.3	3.37	0.1
	C/T - T/T	20 (27.8%)	76 (35.7%)	(0.80–2.59)		(0.75–2.56)		(0.73–15.49)	
rs1065852 (*CYP2D6 *10*)[a]	G/G	48 (66.7%)	132 (62%)	1.23	0.47	1.20	0.55	0.40 (0.09–1.75)	0.23
	G/A - A/A	24 (33.3%)	81 (38%)	(0.70–2.15)		(0.66–2.16)			
rs1080985 (*CYP2D6 *2*)[b]	C/G - G/G	63 (87.5%)	188 (88.3%)	0.93	0.86	0.62	0.45	1.11 (0.47–2.60)	0.82
				(0.41–2.10)		(0.18–2.12)			
	C/C	9 (12,5%)	25 (11,7)						

[a] Dominant model;
[b] Recessive model;
[c] adjusted by covariates;
[d] adjusted by SNPs

Table 4
Analysis of association between SNPs in the population treated with bisoprolol (n = 143) with the event hypotension.

		HYPOTENSION		OR (CI 95%)	p-value	OR (CI 95%)[c]	p-value	OR (CI 95%)[d]	p-value
		YES (n = 38)	NO (n = 105)						
rs1801252 (*ADRB1*; Ser49Gly)[a]	A/A	31 (81.6%)	71 (67.6%)	2.12	0.09	2.69 (0.98–7.41)	0.045	2.14 (0.81–5.69)	0.11
	A/G - G/G	7 (18.4%)	34 (32.4%)	(0.85–5.30)					
rs1801253 (*ADRB1*; Gly389Arg)[b]	C/C - G/C	36 (94.7%)	102 (97.1%)	0.53	0.51	0.23 (0.03–1.85)	0.18	0.77 (0.11–5.20)	0.79
	G/G	2 (5.3%)	3 (2.9%)	(0.08–3.30)					
rs1042713 (*ADRB2*; Gly16Arg)[a]	G/G	11 (28.9%)	46 (43.8%)	0.52	0.1	0.49 (0.20–1.16)	0.09	0.66 (0.25–1.70)	0.38
	G/A - A/A	27 (71%)	59 (56.2%)	(0.23–1.16)					
rs1042714 (*ADRB2*; Glu27Gln)[b]	G/G	1 (2,6%)	16 (15.2%)	0,15	**0.02**	0,05	**< 0.01**	0,17 (0,02–1,56)	0.065
	C/C - G/C	37 (97.4%)	89 (84.8%)	(0,02–0,15)		(0003–0,75)			
rs3892097 (*CYP2D6 *4*)[a]	C/C	27 (71%)	66 (62.9%)	1.45	0.36	1.20 (0.51–2.84)	0.68	1.29	0.82
	C/T - T/T	11 (28.9%)	39 (37.1%)	(0.65–3.24)				(0.14–11.57)	
rs1065852 (*CYP2D6 *10*)[a]	G/G	25 (65.8%)	63 (60%)	1.28	0.53	1.12 (0.49–2.58)	0.79	0.75 (0.10–5.76)	0.78
	G/A - A/A	13 (34.2%)	42 (40%)	(0.59–2.78)					
rs1080985 (*CYP2D6 *2*)[b]	C/G - G/G	34 (89.5%)	93 (88.6%)	1.10	0.88	0.96 (0.27–3.43)	0.95	1.15 (0.31–4.19)	0.83
				(0.33–3.63)					
	C/C	4 (10.5%)	12 (11.4%)						

[a] Dominant model;
[b] Recessive model;
[c] adjusted by covariates;
[d] adjusted by SNPs

Table 5
Predicting results of the variant effect of *ADRB1* and *ADRB2* genes.

SNPs	Gen	Reference allele	Alternative allele	Consecuence	Feature	SIFT	
rs1042713	*ADRB2*	G	A	Missense variant	Protein coding transcripts	0.07	Tolerated
rs1042714	*ADRB2*	G	C			0.36	Tolerated
rs1801252	*ADRB1*	A	G			0.87	Tolerated
rs1801253	*ADRB1*	G	C			1	Tolerated

SNPs: Single Nucleotid Polymorphisms. SIFT: Sorting Intolerant From Tolerant. G: Guanine; A: Adenine; C: Cytosine

4.1. ADRB2 gen (rs1042713, rs1042714)

The most relevant polymorphisms of the *ADRB2 gene*, Gly16Arg and Glu27Gln, are located at the amino-terminal extracellular domain and they influence on the loss of receptor expression, a process known as agonist-mediated downregulation [10]. In this sense, it has been hypothesised that, when the receptors are "hypofunctional", their vasodilator effects are reduced and may therefore be related to hypertension [46,47].

Based on this premise, it has been postulated that the Gly16 (*G*)

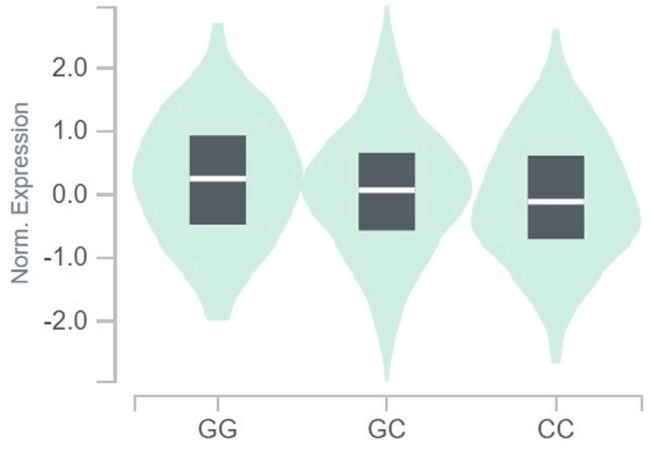

Fig. 1. EQTL analysis – Violin plot showing ADRB2 expression of rs1042714 genotypes in whole blood.

Table 6
Association between ADRB1 and ABRB2 expression and carried genotypes in each analysed tissue.

SNPs	Gen	p-value	Tissues
rs1042713	*ADRB2*	0.056	Whole blood
		0.012	Aortic artery
rs1042714	*ADRB2*	0.00019	Whole blood
rs1801252	*ADRB1*	0.017	Coronary artery
		0.00017	Heart atrial appendix
rs1801253	*ADRB1*	0.032	Aortic artery
		0.046	Coronary artery
		0.0044	Heart atrial appendix

polymorphism may be associated with hypertension [46,50]. As a hypofunctional polymorphism, the expected vasodilator effects of beta 2 receptors would be lower [51,52]. Similarly, the Glu27 (*G*) polymorphism has been associated with a greater increase in BP [53]. However, the results are still controversial.

Sehrt et al. [34] did not find a relevant effect of the Gly16Arg polymorphism on the reduction of BP with carvedilol, but they postulated that the Gly16Arg-Glu27Gln diplotypes could predict the reduction of systolic pressure (SP) linearly proportional to the number of diplotypes. The two SNPs of codons 16 and 27 are linked, Glu27Glu (*GG*) homozygotes are often Gly16Gly (*GG*) homozygotes. The authors also described a greater reduction in BP after carvedilol intake in Gln27 (*C*) allele carriers than in Glu27 (*G*) allele carriers ($p = 0.001$) [46]. Furthermore, *Lanfear et al.* [35], showed that 3-year all-cause mortality was higher in Arg16 or Gln27 carriers in ACS patients treated with BBs. In contrast, *Sounsyrja et al.* [37] and *Filigheddu F et al.* [54] found no differences in BP in hypertensive patients treated with bisoprolol and atenolol, respectively.

In our patients, the rs1042713 (Gly16Arg) SNP was associated with hypotension in the whole population, although the association did not persist after model adjustment. On the contrary, the rs1042714 (Glu27Gln) SNP was associated with hypotension in the whole population, in the cohort of patients treated with bisoprolol and in the cohort of patients treated with other BBs. Glu27Glu (*GG*) homozygous patients may have a lower risk of hypotension than those carrying one or two glutamines; Gln (*C*). In the bisoprolol cohort, the significance value is even higher after adjusting for covariates, whith concomitant treatment with ivabradine increasing the risk of hypotension.

In silico gene expression studies showed significant expression differences in the aorta and in whole blood for the two SNPs, rs1042713 and rs1042714. In both cases, *GG* homozygotes have increased gene expression. These results are consistent with those observed in the association analyses, since a higher expression of *ADRB2* receptors, which have been described as hypofunctional and associated with hypertension, would imply a lower risk of hypotension after taking BBs.

On the other hand, we found no association with the *ADRB2* polymorphisms for bradycardia. In patients with HF, several authors have studied the effect of the *ADRB* genes on the LVEF improvement. *Kaye DM et al.* [33] found that patients with Gln27 (*C*) carriers had a worse response than Glu27 (*G*) carriers and there was no improvement in HF measured as an increase in LVEF$\geq$ 10%. *Metra et al.* [55] found similar results. Among our patients with ACS, 22.8% had HF with LVEF$<$ 50%. In an exploratory manner, the improvement in LVEF $\geq$ 10% was analysed according to the genotype. No significant results were found but, the increase in LVEF $\geq$ 10% after one year of treatment was higher in patients Glu27 carriers than in Gln27Gln homozygotes.

4.2. ADRB1 gene (rs1801252, rs1801253)

Variants in the *ADRB1* gene are common and represent ancestral variation in different populations. The *ADRB1 SNPs*, rs1801252 (Ser49Gly) and rs1801253 (Gly389Arg), have been previously studied for their possible association with CV disease and the response to BBs [27,31].

The combination of both Ser49-Arg389 polymorphisms has been associated with a higher risk of major CV events (death, myocardial infarction, stroke). Some authors previously described that carriers of the Arg389 polymorphism or the Ser49-Arg389 haplotype had a greater reduction in diastolic BP in response to BBs [24,25]. In contrast other authors found no association with the SP response [24,28,31].

In a dose titration study of metoprolol, *Terra SG et al.* [27] described that Gly389 (rs1801253) carriers, unlike Arg389Arg (*CC*) homozygous patients, required other treatments to control HF due to HF decompensation. Therefore, a lack of efficacy in these patients would be considered. Similarly, Ser49Ser (rs1801252) homozygotes also required different HF control treatments compared to Gly49 carriers. In contrast other studies have found no association [21,56].

In our study we found no association between the *ADRB1 SNPs* and bradycardia or hypotension induced by BBs. The SNP rs1801252 (*ADRB1*; Ser49Gly) was in H-W disequilibrium, but we found that this slight imbalance was due to the population size, especially the number of MAF genotype carriers. In the cohort of patients treated with bisoprolol, a trend was observed for Ser49Ser homozygotes to have a higher incidence of hypotensive events than Gly49 carriers. Our results would be in line with those of other authors who, in the last decade, have not found PGx associations for *ADRB1* in patients with cardiac pathologies [21,55,56]. It is therefore likely that *ADRB1* SNPs do not have a relevant influence on the response to BBs, although they may play a role in the risk of CV disease.

4.3. CYP2D6 gene (CYP2D6*4, CYP2D6*10, CYP2D6*2)

In the Caucasian population, between 5% and 10% are PM, due to the inheritance of two non-functional alleles (*CYP2D6*3, *4, *5, *6*), of which *CYP2D6 * 4* is the most common in this population [57]. *CYP2D6*10* is considered a reduced function allele, whereas *CYP2D6*2* is considered a normal function allele.

In our study, we only found an initial association for *CYP2D6*10* with the bradycardia event in the entire cohort of patients. Although the association was maintained after adjustment for covariates, it did not persist after Bonferroni correction. Wild type (*GG*) patients with normal phenotype metabolism presented more bradycardia events than heterozygotes (*GA*) with IM phenotype and homozygous recessive (*AA*) with PM phenotype. In the population of bisoprolol and other BBs separately, we found no significant results.

Recently, in a study of hypertensive patients in China, the authors observed that *CYP2D6* and *CYP3A5* did not affect the plasmatic concentration of bisoprolol and were not correlated with BP reduction [58].

The bisoprolol kinetics genome-wide association study (GWAS) by *Fontana V et al.* [5] did not show association, although both enzymes participate in the metabolism of bisoprolol. The results of our study, in agreement with those of other authors [34,59,60], suggest that *CYP2D6* is not associated with the incidence of AEs. To date, there are no DPWG dosing recommendations for bisoprolol based on *CYP2D6* genotype [61].

4.4. Limitations

This study has several limitations. It is an observational study; this means that we did not assess the clinical impact of the genetic polymorphisms studied on the response of BBs in everyday clinical conditions.

We included several BBs in the first association analysis that could be affected by *CYP2D6* in different ways during metabolism. We could not collect the basal BP and HR parameters to analyse the variation of the parameters, because the patients were recruited after hospital admission for the ACS and these parameters are altered during the first days of admission.

Similarly, ACS patients undergoing PCI-stent, especially in elderly individuals, often rely on a combination of medications that can significantly impact their health outcomes. While our association analyses take into account the direct effects of concomitant medication, it is important to recognise that the pharmacogenetics of these drugs may also play a role in shaping patient outcomes and the possible influence of genetic polymorphisms affecting other drugs response should be taking into account [62]. Fortunately, in this study, it was possible to study the influence of genetic polymorphisms affecting the response to antiplatelet drugs, since all patients received antiplatelet treatment based on *CYP2C19/ABCB1* pharmacogenetic testing, thus minimising the effect of variants that affect the response to antiplatelet drugs [63].

Of all the drugs prescribed for the type of patient with ACS-PCI-stent, BBs may have the highest incidence of bradycardia. So the results obtained in this study may be largely due to the influence of the pharmacogenetics of BBs. But with respect to hypotension, other drugs can produce hypotension (antihypertensives.) and this variable has not been taken into account.

5. Conclusions

Some genetic variants may be associated with bradycardia and hypotension in patients treated with BBs. In patients with ACS, the *CYP2D6*10* (rs1065852) polymorphism initially showed an association with bradycardia in the overall population of this study, but its influence on the response to the studied BBs does not seem relevant. The *CYP2D6*4* (rs3892097) and *CYP2D6 * 2* (rs1080985) polymorphisms were not associated with tolerance to BBs either overall or in the population of bisoprolol and other BBs. The *ADRB1* polymorphisms (rs1801252 and rs1801253) included in this study were not shown to be associated with BB tolerance measured as bradycardia or hypotension. In this study we found strong associations between SNPs in *ADRB2* and BB-induced hypotension. The presence of the G allele in *ADRB2* (rs1042714; Glu27Gln) and the *GG* genotype conferred a protective effect against hypotension by treatment with BBs. The *ADRB2* (rs1042713; Gly16Arg) *GG* genotype may also confer a protective effect against the occurrence of hypotension induced by BBs. Future studies exploring PGx associations with BBs should focus on the influence of *ADRB2*.

Ethic disclosure

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Granada (Spain) "CEIM/CEI Provincial de Granada". Informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Celia Castaño-Amores: Conceptualization, Methodology, Writing – original draft, Investigation, Resources, Visualization. **Alba Antúnez-Rodríguez:** Writing – review & editing, Formal analysis, Data curation. **Ana Pozo-Agundo:** Writing – review & editing, Data curation. **Sonia García Rodríguez**: Data analysis and review. **Luis Javier Martínez-González:** Validation, Writing – review & editing, Supervision. **Cristina Lucía Davila-Fajardo:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration. *All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115869.

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