

Association of β_1 and β_2 -adrenoceptor Polymorphisms With the Demand of Inotropic Catecholamine Support Following Coronary Artery Bypass Grafting in Iranian Population

Gohar Eslami

Mazandaran University of Medical Sciences

Rahman Ghafari

Mazandaran University of Medical Sciences

Valiollah Habibi

Mazandaran University of Medical Sciences

Akbar Hedayatizadeh-Omran

Mazandaran University of Medical Sciences

Mahmood Moosazadeh

Mazandaran University of Medical Sciences

Omolbanin Amjadi

Mazandaran University of Medical Sciences

Reza Alikhani

Mazandaran University of Medical Sciences

Shahaboddin Emami (✉ sha_emami@yahoo.com)

Pharmaceutical Research Center, Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran

<https://orcid.org/0000-0003-4739-5313>

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Abstract

Background: Hemodynamic instability is a common complication in the first hours following cardiac surgery and inotropic catecholamine support is an acceptable treatment strategy for its management. β_1 and β_2 -adrenoceptors (β_1 and β_2 AR) are mediated the positive inotropic and chronotropic responses of the heart to catecholamines. Previous evidence has suggested an association between β_1 and β_2 AR polymorphisms and cardiac response and change in receptor signaling. The aim of this study was evaluating the relationship between β_1 and β_2 AR polymorphisms with demand of catecholamine inotropic support among coronary artery bypass grafting (CABG) patients.

Methods: One hundred ninety-eight consecutive patients who underwent CABG with cardiopulmonary bypass were included in this study. We assessed hemodynamic parameters, dose and duration of inotropic support according to β_1 and β_2 AR genotypes in post-operative period. DNA genotyping were assessed through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and PCR genotyping results were confirmed by direct DNA sequencing.

Results: The our results indicated that patients carrying one or two alleles of the Arg389- β_1 AR variant required significantly shorter inotropic support time compared with patients homozygous for the Gly389- β_1 AR ($p=0.003$). Finally, neither β_1 AR polymorphisms nor Arg16Gly- β_2 AR polymorphism are associated with catecholamines-induced hemodynamic effects.

Conclusion: These findings suggest that genetic variability in the β_1 and β_2 AR polymorphisms may not be a major determinant of cardiac responses to catecholamine treatment in Iranian population. However, larger scale studies with different ethnicities are needed for confirmation.

Background

Hemodynamic disturbances and impaired cardiac functions are major problems and cause of death following coronary artery bypass grafting (CABG) surgery with cardiopulmonary bypass (CPB) that is secondary to myocardial hypoxia or ischemia [1]. The condition is manifested by cardinal signs such as hypotension, peripheral vasoconstriction and oliguria. The mechanism of post-operative hypotension is incompletely understood. It is believed that systemic inflammation and vasoplegia that occur in patients following major cardiac surgery manifest as systemic arterial vasodilation and profound hypotension with a high cardiac index and a low systemic vascular resistance [2]. Inotropic support by catecholamines are the cornerstone treatment of hypotensive post-CABG states to improve blood pressure and organ perfusion via increasing cardiac output with effect on beta and alfa adrenergic receptors. However, in many patients who experience post-CABG depressed myocardial function, inadequate and weak response to catecholamines is observed and identification of patients who will adequately respond to inotrope therapy remains a challenge [3].

β_1 -adrenergic pathway through positive inotropic and chronotropic effects, play a key role in the regulation of heart rate and contractility and is responsive to the effects of circulating catecholamines [4, 5]. In addition, β_2 -adrenoceptors (β_2 ARs) are also expressed on the myocardium, atria of the heart and in vascular smooth muscle beds. β_2 ARs are allowed for calcium influx in response to cardiac sympathetic nerve activity, leading to positive inotropic and chronotropic effects [6, 7]. Recent in vivo and in vitro studies have shown multiple genetic variants in β_1 and β_2 ARs [8, 9]. This implies that variations in the β_1 and β_2 AR gene might be explain some variability observed in response of patients CABG surgery to inotropic support therapy.

Enhanced left ventricular ejection fraction and also greater stroke volume, cardiac output and mean arterial pressure has been observed in healthy subjects homozygous for the Gly16- β_2 AR allele [10, 11]. Additionally, previous studies (Bruck et al., 2005, La Rosée et al., 2004) have demonstrated a higher heart rate and/or contractility among individuals homozygous for the Arg389- β_1 AR variant compared with other codon 389 genotype carriers [12, 13]. A study done by Leineweber et al., 2007, in sample of German population showed that less post-surgical inotropic support is required in patients undergoing CABG who were preoperatively chronically treated with metoprolol and homozygous for the Arg389- β_1 AR, than those with one or two Gly389- β_1 AR alleles [14]. Over-responsiveness to propranolol has been reported in Iranian population that may be associated with polymorphism of β -adrenoceptors [15]. Furthermore, it was observed that Arg16Gly- β_2 AR genotype may have protective effect for hypertension in comparison with Arg16Arg- β_2 AR variant in Iranian population [16].

Therefore, the present study was designed to investigate whether the hemodynamic responses to inotropic support by epinephrine in Iranian patients with hemodynamic instability following CABG surgery with CPB are influenced by β_1 and β_2 AR polymorphic variations.

Methods

Study population

This was a prospective study included 198 patients attending Mazandaran Heart Center, Sari, Iran between April and September 2018. The patients aged 18 years or older and indicated for elective CABG surgery with CPB that received inotropic support only by epinephrine after weaning from CPB or in the first 12 hours after post-operative ICU admission were included in this study. The patients who needed a pacemaker, intra-aortic balloon pump after weaning from CPB, those who also had post-operative major bleeding (> 200 ml/h first 6 h after surgery), septic shock, and preoperative end-stage renal disease or post-operative severe renal failure (creatinine clearance < 30 ml/min/1.73 m²) requiring temporary hemodialysis were also excluded from the study. The study protocol was approved by the ethics committee of Mazandaran University of Medical Sciences.

Data collection and clinical assessment

Pre and post-operative demographics, medical history and operative details were extracted from patient's medical records. Preoperatively, the usual doses of cardiac medications were prescribed for all participants and none received inotropic support.

A systolic blood pressure (SBP) < 90 mmHg or mean arterial pressure (MAP) < 65 mmHg was considered as the post-operative hypotension that required inotropic support. Inotropic support by epinephrine was started at the rate of 10 ng/kg/min and titrated against blood pressure to achieve hemodynamic target of mean arterial pressure between 70 and 90 mmHg.

At the time when the inotrope infusion was initiated, systolic and diastolic blood pressure (DBP), mean arterial pressure, heart rate (HR), central venous pressure (CVP) and urine output were recorded. During the inotrope infusion, these parameters were frequently monitored. In addition, the required dose and total duration of epinephrine infusion were recorded. In order to maintain adequate circulating volume and electrolyte balance during inotrope administration, all patients received intravenous isotonic fluids.

DNA extraction

Five milliliters of peripheral venous blood samples were taken in tubes containing ethylene diamine tetraacetic acid (EDTA) and preserved at -80 °C for isolation of DNA. Genomic DNA was extracted using a column-based DNA isolation kit (Denazist Asia Co., Mashhad, Iran) according to the manufacturer's instructions. The concentration and purity of the extracted DNA were measured using a Nanodrop spectrophotometer (Biochrom WPA Biowave II⁺ UV/visible spectrophotometer, Cambridge, UK).

Genotyping using PCR-RFLP method

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed to determine the β_1 AR gene polymorphisms (codon 389, nucleotide 1165 and codon 49, nucleotide 145) and β_2 AR gene polymorphism (codon 16, nucleotide 46). PCR was performed using 200 ng of template DNA, 10 μ l Taq DNA polymerase 2x master mix RED (Ampliqon A/S, Odense, Denmark) containing 150 mM Tris-HCl pH 8.5, 40 mM (NH₄)₂SO₄, 3 mM MgCl₂, 0.2% Tween 20, 0.4 mM of each dNTP and 0.2 U/ μ l Ampliqon Taq DNA polymerase, along with 10 pmol of specific primers, and 7 μ l nuclease-free water. The PCR condition, sequence of primers and expected product lengths are listed in Table 1. The PCR products were restriction-digested with 10 U/ μ l of *BcgI* (β_1 AR codon 389), *EcoO109I* (β_1 AR codon 49) (Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C for 3 hours and *BsrDI* (β_2 AR codon 16) (Bio Lab; New England) at 55°C for 3 hours. Digested fragments were analyzed by electrophoretic separation on 2% agarose gel containing DNA green fluorescent dye (DenaZist, Mashhad, Iran) (Fig. 1). The staffs of the ICU and the investigators were blinded to the genotypes of the patients.

Table 1 PCR^a condition and characteristics of polymorphisms, primers used, and length of digested products

SNP ^b / rs name	Primers sequence (5'→ 3')	PCR product size	PCR conditions	Length of digested products
Arg389Gly rs1801253	F ^c : CATCATGGGCGTCTTCACGC R ^d : TGGGCTTCGAGTTCACCTGC	547 bp	98 °C: 3min, followed by 35 cycles at 98 °C: 30s, 60 °C:1min, 72 °C:30s	GG: 547 GC: 547,342,171,34 CC: 342,171,34
Ser49Gly rs1801252	F: CCGGGCTTCTGGGGTGTTC R: GGCGAGGTGATGGCGAGGTAGC	562 bp	98 °C: 3min, followed by 35 cycles at 98 °C: 45s, 60 °C:1min, 72 °C:30s	AA: 562 AG: 562,343,219 GG: 343,219
Arg16Gly rs1042713	F: CTTCTTGCTGGCACGCAAT R: CCAGTGAAGTGATGAAGTAGTTGG	200 bp	94 °C: 3min, followed by 35 cycles at 94 °C: 1min, 54 °C:1min, 72 °C:1min, 72 °C: 5min	GG: 108,56,23,14 GA: 131,108,56,23,14 AA: 131,56,14

a Polymerase chain reaction, b Single nucleotide polymorphism, c Forward primer, d Reverse primer

PCR products sequencing

The genotypes determined by PCR-RFLP were further confirmed by direct sequencing (Fig. 1). Genotyping was performed without knowing the population study status. Ten percent of random samples were tested by Sanger sequencing with forward and reverse primers by ABI 3130XL.

Statistical analysis

The data were analyzed by SPSS Statistics software, version 22 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were quoted as mean ± standard deviation (SD) and the results on the categorical measurements were expressed as numbers and percent. Chi-square test and Fisher's exact test were used for categorical variables. Differences between means were compared using the one-way analysis of variance (ANOVA). Post-hoc analysis was performed when ANOVA was indicated significance. The Hardy-Weinberg equilibrium also was tested by the chi-square test to determine the frequency distribution of genotypes. P<0.05 was considered as statistical differences in each test.

Results

The mean age of patents was 62.1 ± 10.1 years (65.7% male). One hundred ninety-eight patient samples were genotyped for Arg389Gly-β₁AR (rs1801253) and Ser49Gly-β₁AR (rs1801252) polymorphisms and 162 patient samples for Arg16Gly-β₂AR (rs1042713) polymorphism.

Among all participants, the allele frequency for the Gly389-β₁AR and Ser49-β₁AR alleles were 53% and 90%, respectively and also for Arg389-β₁AR and Gly49-β₁AR alleles were 47% and 10%, respectively. Furthermore, the frequency of Arg16-β₂AR and Gly16-β₂AR alleles were 15.7% and 84.3%, respectively. Regarding the genotypes found in our population, 52 patients (26.3%) undergoing CABG surgery with CPB were homozygous for Gly at position 389 (Gly389Gly), 105 patients (53%) heterozygous for Arg389Gly and 41 (20.7%) homozygous for Arg at position 389 (Arg389Arg). The genotype frequencies of Ser49Gly-β₁AR polymorphism was Ser49Ser (80.3%), Ser49Gly (19.2%) and Gly49Gly (0.5%). Moreover, genotyping revealed a prevalence of homozygous patients for Arg16Arg of 6.8%, heterozygous for Arg389Gly of 17.9% and homozygous for Gly389Gly of 75.3%.

Baseline demographics, operative details and post-operative clinical information of patients according to by β₁ and β₂AR genotypes are listed in Tables 2 and 3, respectively. Baseline characteristics were similar in all variants (Table 2). As illustrated in Table 3, Operative

details and post-operative data were similar between the study population with different polymorphisms. However, regarding the Arg16Gly- β 2AR polymorphism, post-operative left ventricular ejection fraction (LVEF) was significantly higher in patients with the Arg16Arg genotype with $55 \pm 5.5\%$ versus $48.5 \pm 9.2\%$ in patients with Arg16Gly and $51.1 \pm 7\%$ in Gly16Gly carriers ($p = 0.028$). There was no statistically significant difference in post-operative LVEF between the two β_1 AR polymorphisms.

Table 2

Baseline characteristics of patients according to Arg389Gly- β_1 AR, Ser49Gly- β_1 AR and Arg16Gly- β_2 AR polymorphisms

Characteristics	Gly389Gly (n = 52)	Arg389Gly (n = 105)	Arg389Arg (n = 41)	Ser49Ser (n = 159)	Ser49Gly (n = 38)	Gly49Gly (n = 1)	Arg16Arg (n = 11)	Arg16Gly (n = 29)	Gly16Gly (n = 122)
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a *Body mass index*, b *Left ventricular ejection fraction*, c *Creatinine clearance*, d *Myocardial infarction*, e *Chronic obstructive pulmonary disease*, f *Angiotensin-converting enzyme*, g *Angiotensin receptor blocker*, h *Heart rate*, i *Systolic blood pressure*, j *Diastolic blood pressure*, k *Mean arterial pressure*

Values are presented as mean \pm SD where appropriate

Not significant for all variables

Characteristics	Gly389Gly (n = 52)	Arg389Gly (n = 105)	Arg389Arg (n = 41)	Ser49Ser (n = 159)	Ser49Gly (n = 38)	Gly49Gly (n = 1)	Arg16Arg (n = 11)	Arg16Gly (n = 29)	Gly16Gly (n = 122)
Sex (male/female), n	34/18	70/35	26/15	105/54	24/14	1/0	8/3	14/15	85/37
Age (year)	62.1 ± 10.4	62.8 ± 9.9	60.4 ± 10.2	62.3 ± 10.1	62.1 ± 9.4	47	62.4 ± 9.3	60.3 ± 11.1	62.3 ± 9.7
BMI ^a (kg/m ²)	26.9 ± 4.3	26.8 ± 4.3	27.7 ± 3.6	27 ± 4.3	26.9 ± 3.5	28	26.6 ± 4.2	26.5 ± 4.6	26.9 ± 4
EuroScore II	2.1 ± 1.5	2 ± 1.7	1.6 ± 1.1	2 ± 1.7	1.7 ± 0.9	1 (100%)	1.5 ± 0.9	2.4 ± 1.9	1.9 ± 1.4
Smoking, n	14 (26.9%)	30 (28.6%)	12 (29.3%)	45 (28.3%)	10 (26.3%)	50	2 (18.2%)	11 (37.9%)	35 (28.7%)
Preoperative LVEF ^b (%)	51.3 ± 6.6	52.2 ± 6.7	53.9 ± 5.6	52.5 ± 6.5	51.5 ± 6.7	117	53.6 ± 6.7	50.3 ± 6.7	52.5 ± 6.4
Clcr ^c (ml/min/1.73 m ²)	71.9 ± 22.5	71.2 ± 20.2	78.9 ± 20.1	72.4 ± 20.8	74.4 ± 20.5	0	73.4 ± 21.1	70.9 ± 21.5	72.7 ± 21
Comorbid features, n	35 (67.3%)	66 (62.9%)	29 (70.7%)	25 (15.7%)	5 (13.2%)	0	2 (18.2%)	5 (17.2%)	81 (66.4%)
Previous MI ^d	26 (50%)	39 (37.1%)	14 (34.1%)	106 (66.7%)	24 (63.2%)	0	5 (45.5%)	22 (75.9%)	50 (41%)
Hypertension	22 (42.3%)	53 (50.5%)	16 (39%)	67 (42.1%)	12 (31.6%)	1 (100%)	6 (54.5%)	11 (37.9%)	59 (48.4%)
Diabetes mellitus	1 (1.9%)	4 (3.8%)	2 (4.9%)	76 (47.8%)	15 (39.5%)	0	4 (36.4%)	11 (37.9%)	6 (4.9%)
Dyslipidemia	43 (82.7%)	95 (90.5%)	34 (82.9%)	5 (3.1%)	2 (5.3%)	0	0	1 (3.4%)	104 (85.2%)
COPD ^e	21 (40.4%)	55 (52.4%)	19 (46.3%)	140 (88.1%)	31 (81.6%)	0	9 (81.8%)	26 (89.7%)	62 (50.8%)
Medication status, n	17 (32.7%)	30 (28.6%)	12 (29.3%)	83 (52.2%)	16 (42.1%)	132	2 (18.2%)	13 (44.8%)	35 (28.7%)
β-blocker	18 (34.6%)	32 (30.5%)	12 (29.3%)	45 (28.3%)	13 (34.2%)	83	3 (27.3%)	9 (31%)	37 (30.3%)
ACE ^f inhibitor	1 (1.9%)	1 (1%)	3 (7.3%)	50 (31.4%)	12 (31.6%)	99.3	4 (36.4%)	9 (31%)	3 (2.5%)
ARB ^g	70.9 ± 9.7	70.3 ± 8.5	68.7 ± 10.4	3 (1.9%)	1 (2.6%)	0	0	70 ± 7.5	70.5 ± 9.3
Diuretic	129.5 ± 9.4	129.8 ± 8	132.6 ± 7.3	70.3 ± 9.4	69.1 ± 8.3	0	70 ± 11.2	129.6 ± 9.2	129.8 ± 8.4
Digoxin	81 ± 4.4	81.4 ± 4.4	82.4 ± 4.8	129.8 ± 7.7	132.5 ± 10.4	0	126.5 ± 5.9	80.7 ± 3.7	81.5 ± 4.7
Hemodynamic status (before surgery)	97.2 ± 5.3	97.5 ± 5.2	99.1 ± 4.3	81.4 ± 4.5	81.8 ± 4.3	0	78.5 ± 3.5	97 ± 5	97.6 ± 5.1
HR ^h (beats/min)				97.5 ± 5	98.7 ± 5.6	0	94.5 ± 4.1		
SBP ⁱ (mmHg)									
DBP ^j (mmHg)									

a Body mass index, b Left ventricular ejection fraction, c Creatinine clearance, d Myocardial infarction, e Chronic obstructive pulmonary disease, f Angiotensin-converting enzyme, g Angiotensin receptor blocker, h Heart rate, i Systolic blood pressure, j Diastolic blood pressure, k Mean arterial pressure

Values are presented as mean ± SD where appropriate

Not significant for all variables

Characteristics	Gly389Gly	Arg389Gly	Arg389Arg	Ser49Ser	Ser49Gly	Gly49Gly	Arg16Arg	Arg16Gly	Gly16Gly
MAP ^k (mmHg)	(n = 52)	(n = 105)	(n = 41)	(n = 159)	(n = 38)	(n = 1)	(n = 11)	(n = 29)	(n = 122)
<i>a Body mass index, b Left ventricular ejection fraction, c Creatinine clearance, d Myocardial infarction, e Chronic obstructive pulmonary disease, f Angiotensin-converting enzyme, g Angiotensin receptor blocker, h Heart rate, i Systolic blood pressure, j Diastolic blood pressure, k Mean arterial pressure</i>									
Values are presented as mean ± SD where appropriate									
Not significant for all variables									

Table 3

Operative details and post-operative data of patients according to Arg389Gly-β₁AR, Ser49Gly-β₁AR and Arg16Gly-β₂AR polymorphisms

Characteristics	Gly389Gly	Arg389Gly	Arg389Arg	Ser49Ser	Ser49Gly	Gly49Gly	Arg16Arg	Arg16Gly	Gly16Gly
	(n = 52)	(n = 105)	(n = 41)	(n = 159)	(n = 38)	(n = 1)	(n = 11)	(n = 29)	(n = 122)
LMCA ^a disease, n	7 (13.5%)	15 (14.3%)	4 (9.8%)	22 (13.8%)	3 (7.9%)	1 (100%)	0	3 (10.3%)	17 (13.9%)
Grafts per patient, n	3.5 ± 0.9	3.4 ± 0.8	3.3 ± 0.6	3.4 ± 0.8	3.5 ± 0.6	3	3.3 ± 0.6	3.2 ± 0.7	3.4 ± 0.8
Operation time (h)	4.1 ± 0.9	4 ± 1	4 ± 0.8	4.1 ± 0.9	3.8 ± 0.8	4	4.6 ± 1	4.2 ± 1.3	4 ± 0.8
CCT ^b (min)	62.5 ± 32.7	57.4 ± 28.2	54.2 ± 21.9	59.6 ± 29.8	52.6 ± 20.4	31	73.4 ± 46.3	66.6 ± 28.2	56.2 ± 27.9
CPBT ^c (min)	97 ± 43.4	89 ± 42	89.3 ± 39.6	93.1 ± 44.6	83.5 ± 27	50	113.9 ± 63.5	108.1 ± 57.8	88.3 ± 37.4
Post-operative LVEF ^d (%)	15.3 ± 8.2	50.8 ± 8	51.2 ± 7.8	50.9 ± 7.9	14 ± 6.7	16	55 ± 5.5*	48.5 ± 9.2	51.1 ± 7
Time on MV ^e (h)		15.1 ± 8.3	12.4 ± 6.9	14.5 ± 8			16.2 ± 8.6	16.5 ± 8.1	14.5 ± 8.6
<i>a Left main coronary artery, b Cross-clamp time, c Cardiopulmonary bypass time, d Left ventricular ejection fraction, e Mechanical ventilation</i>									
Values are presented as mean ± SD where appropriate									
*p < 0.05 vs. other variants (p = 0.028)									

Hemodynamic variables and changes in hemodynamic parameters as compared to baseline after the initiation of epinephrine infusion are presented in Table 4 and Fig. 2. The results showed that increases in SBP, DBP, MAP and HR were similar after inotropic support by epinephrine and there were no significant differences between the three polymorphisms studied. Similar results were also obtained for decrease in CVP according to β₁ and β₂AR genotypes. Although Gly389Gly-β₁AR and Gly16Gly-β₂AR homozygous and Ser49Gly-β₁AR heterozygous patients had a higher changes in HR compared to other variants, no significant differences were found between the three βAR polymorphisms. Furthermore, the increase in SBP and MAP and decrease in CVP were, in patients heterozygous for the Ser49Gly-β₁AR variant, higher than patients with other variants. However, these changes were small and not statistically significant (Table 4, Fig. 2). As can be seen from Fig. 2, in patients carrying one or two Arg alleles at codon 389, the duration of inotropic support after CABG surgery with CPB were significantly shorter (P < 0.05) than patients homozygous for the Gly389-β₁AR. There was a trend towards lower dose of epinephrine required to achieve the hemodynamic goal in patients homozygous for the Arg389-β₁AR, Ser49-β₁AR and Gly16-β₂AR variants compare to other genotypes, although this difference just failed to reach statistical significance.

Table 4

Hemodynamic status of patients before initiation of inotropic support and changes induced by inotropic support according to Arg389Gly- β_1 AR, Ser49Gly- β_1 AR and Arg16Gly- β_2 AR polymorphisms

	Gly389Gly (n = 52)	Arg389Gly (n = 105)	Arg389Arg (n = 41)	Ser49Ser (n = 159)	Ser49Gly (n = 38)	Gly49Gly (n = 1)	Arg16Arg (n = 11)	Arg16Gly (n = 29)	Gly16Gly (n = 122)
HR ^a (beats/min)	87.6 ± 13.6	86.4 ± 10.8	88.1 ± 12.8	86.6 ± 11.7	87.9 ± 11.8	88	83.3 ± 12.1	85.7 ± 11.9	87.9 ± 11.8
Baseline	98.9 ± 12.1	95.2 ± 11.9	96.2 ± 12.2	96.7 ± 12.2	94.2 ± 11.7	98	89.4 ± 12.1	95.3 ± 13.9	97.8 ± 11.1
Response	80.5 ± 7.7	80.6 ± 6.2	81.5 ± 4.7	81.1 ± 6.5	78.1 ± 5.9	108	82.8 ± 5.9	80.8 ± 4.8	80.4 ± 6.9
SBP ^b (mmHg)	105.5 ± 7.9	106.7 ± 8.2	107.1 ± 10.1	106.3 ± 7.9	107.1 ± 10.9	48	107 ± 9.6	105.9 ± 9.7	106.5 ± 7.9
Baseline	48.7 ± 4.2	48.2 ± 5.1	48.5 ± 3.9	48.5 ± 4.9	47.3 ± 3.4	51	50.1 ± 2.3	47 ± 4.8	48.5 ± 4.8
Response	57.9 ± 3.2	58.8 ± 5.6	59.4 ± 5.2	58.4 ± 4.6	60.3 ± 6.4	72	57.3 ± 3.7	57.2 ± 4.3	59.3 ± 5.2
DBP ^c (mmHg)	70.1 ± 6.3	69.9 ± 5.1	70.5 ± 3.2	70.4 ± 5.3	67.9 ± 4.4	89	71.9 ± 4.1	69.9 ± 4.4	69.8 ± 5.6
Baseline	89.6 ± 5.5	90.7 ± 6.6	91.2 ± 8.2	90.3 ± 6.2	91.5 ± 8.6	16	90.4 ± 6.8	89.6 ± 7.4	90.8 ± 6.3
Response	14.8 ± 3.6	15.6 ± 3.2	14.6 ± 3.9	15.1 ± 3.3	16.4 ± 4.1	12	13.7 ± 4.1	14.7 ± 3.1	15.6 ± 3.4
MAP ^d (mmHg)	10.6 ± 4.7	12.1 ± 4.5	10.5 ± 5.1	11.3 ± 4.5	12.1 ± 5.6		9.9 ± 5.2	11.1 ± 4.8	11.7 ± 4.5
Baseline									
Response									
CVP ^e (cm H ₂ O)									
Baseline									
Response									

a Heart rate, b Systolic blood pressure, c Diastolic blood pressure, d Mean arterial pressure, e Central venous pressure

Values are presented as mean ± SD

Not significant for all variables

Discussion

Poor cardiac function and post-operative hypotension during the early hours after CABG surgery with CPB is associated with a number of serious complications including vasospasm, generalized inflammatory response, organ dysfunction and an increased risk of death [17]. Epinephrine is an effective initial inotropic agent that widely used in patients with hypotension following cardiac surgery and clearly improves cardiac output and myocardial performance [18]. However, among post-cardiac surgery patients different responses to inotropic agents were observed. Beta-adrenoceptors mediate the physiological effects of catecholamines and also these receptors are polymorphic. Hence, inter-individual differences in genetic composition could lead to various drug responses and it could give rise to innovating effective treatment approaches. Although previous studies (Leineweber et al., 2007, Dhein et al., 2017) have proven the significant association of β_1 AR gene and its polymorphisms in cardiovascular diseases and their impact on responses to β AR agonist treatment, still its role as therapeutic determinant remains unclear [14, 19]. According to our knowledge, there have been neither previous investigations of the genetic basis of hemodynamic effects of positive inotropic agents in patients undergoing CABG surgery with CPB in Iranian population. Results of our study suggest lack of any association between the three polymorphisms studied and patient's hemodynamic response to inotropic support after CABG surgery with CPB. In addition, patients carrying one or two Arg389- β_1 AR alleles needed significantly less inotropic support time compared to Gly389- β_1 AR homozygotes.

Evidence suggests that mutant Arg389Arg- β_1 AR is a known genetic risk factor for cardiovascular diseases [8, 20]. In the present study, we found 41 (20.7%) patients were carrying mutant Arg389Arg- β_1 AR genotype, which is lower than the reported frequency in other white populations [4, 19, 21]. Regarding the genotypes found in our population, the homozygous Gly49Gly- β_1 AR variant was observed only in one patient. The Gly49Gly variant is extremely rare and its lower frequency was also reported in previous studies within various ethnic

populations (22–25). Furthermore, in good accordance with our data, Gly16- β_2 AR and Gly16Gly- β_2 AR were reported as the most prominent alleles and genotypes among different ethnic populations (White and Black Americans, Chinese and Egyptian populations) [26, 27].

The Gly389- β_1 AR has a 3-4-fold fewer isoprenaline stimulation of the adenylyl cyclase than the Arg389- β_1 AR variant [12]. Accordingly, patients carrying the Arg389- β_1 AR allele would be expected more responsive to inotropic therapy and also need lower doses of catecholamine than others. This is in accordance with the findings of a study by Dhein et al., 2017, that also reported more norepinephrine requirements in patients with Gly389 variant of the β_1 AR compared to those carrying one or two Arg389- β_1 AR alleles [19]. In addition, Leineweber et al., 2007, also found that the Gly389Gly- β_1 AR variant was significantly associated with higher required dose of epinephrine to reach stable and comparable hemodynamic response [14]. However, the results of our study were in disagreement with data obtained from above-mentioned studies. The present data showed that the dose of epinephrine needed for improvement of cardiac function in patients homozygous for Arg389- β_1 AR was two times higher than for subjects homozygous for Gly389- β_1 AR. On the other hand, patients who carried Gly389Gly- β_1 AR genotype responded two times more to inotropic support therapy in comparison with patients homozygous for Arg at codon 389, although this just failed to reach statistical significance ($p = 0.703$). According to our finding, it may be postulated that there is an Arg389Gly- β_1 AR gene-dose effect.

It was reported that the time of inotropic support was the shortest in patients homozygous for the Arg allele at codon 389, compared to patients homozygous for the Gly389- β_1 AR variant [14]. This is in good accordance to present data. Our results demonstrated that the time of inotropic support to achieve positive inotropic response was significantly shortest in Arg389Gly- β_1 AR heterozygous and Arg389Arg- β_1 AR homozygous patients ($p = 0.003$). Furthermore, comparing with the reported inotrope infusion time in Leineweber et al., 2007 study, the mean inotropic support time was relatively shorter in current study (2.71 vs 20.5 hours in Arg389Arg- β_1 AR and 6 vs 10.5 hours in Gly389Gly- β_1 AR) [14].

Present findings did not show any statistically significant association between hemodynamic measurements (SBP, MAP, CVP, HR) and β_1 AR variants at codon 389 in patients receiving inotropic support ($p = 0.0.922$, $p = 0.807$, $p = 0.974$ and $p = 0.274$, respectively). Similarly, the influence of the Arg389Gly- β_1 AR polymorphism on modified hemodynamic responses to epinephrine and norepinephrine in young healthy adults was not observed [28, 29]. It is worth noting that several studies on the importance of genetic variations of β_1 AR on hemodynamic responses yielded conflicting results. In this context, in a study (Bruck et al., 2005) comparing cardiac responses to β_1 AR stimulation by rather β_1 AR selective agonist dobutamine in healthy subjects, improvement of cardiac function was superior in the cardiac contractility and blood pressure in individuals carrying the Arg389Arg- β_1 AR homozygous than the patients carrying the Gly389Gly- β_1 AR variant [12]. Moreover, administration of dobutamine in subjects homozygous for the Arg389- β_1 AR caused significantly larger increases in heart rate compared to carriers of one or two alleles of the Gly389- β_1 AR [8, 13]. These differences may be postulated that inter-individual variations in required time to improve cardiac function and hemodynamic response to inotropic support after cardiac surgery might be in association with other different genetic mutations and variations of adrenergic system.

According to the results of our study, the Ser49Gly- β_1 AR polymorphism has no significant influence on SBP, MAP, CVP and HR ($p = 0.505$, $p = 0.309$, $p = 0.859$ and $p = 0.179$, respectively). This is consistent with the findings Kumar et al., 2008, who found that Ser49Gly- β_1 AR polymorphism did not alter the cardiac response and also systolic and diastolic blood pressures among South Indians [24]. Ser49Ser- β_1 AR was found to be associated with a higher mean HR under resting conditions, which might result in a higher heart rate as we observed for Ser49Ser- β_1 AR genotype carriers, although this just failed to reach statistical significance ($p = 0.179$) (Fig. 2) [30]. In our inotropic support patients with Ser49Ser- β_1 AR variant, a lower dose of epinephrine was required to achieve positive inotropic response than Ser49Gly- β_1 AR genotype. Meanwhile, the Gly49 variant of the β_1 -adrenoceptor gene was shown to be associated with much higher desensitization and faster down-regulation of the β_1 -adrenoceptors [31–33]. On the other hand, in current study, patients with the Ser49Ser- β_1 AR variant responded better to the inotropic support than those carrying Gly49- β_1 AR allele; however, there was no significant difference between the two variant carriers ($p = 0.831$).

In vitro studies exhibited that the Gly16- β_2 AR allele enhanced β AR down-regulation [26]. In accordance with this theory, this down-regulation would be expected to decrease heart rate and also attenuating the effects of inotropic and chronotropic of catecholamines. This might result in higher required dose of epinephrine in patients carrying one or two Gly16- β_2 AR alleles as observed in our study. By contrast, present findings demonstrated that increase in heart rate in response to β AR stimulation by epinephrine in Arg16Arg- β_2 AR patients was less than other β_2 AR variants. However, we did not find any association between the Arg16Gly- β_2 AR polymorphism and increases in heart rate ($p = 0.314$). This was in line with previous studies performed in young healthy subjects that failed to find any significant genotype-dependent differences between homozygous for the Arg16 or Gly16- β_2 AR in male volunteers with increases in heart rate and contractility

induced by terbutaline infusion [34, 35]. Down-regulation of β_2 AR genotype may occur genotype independently at a similar level among all patients; this would explain a similar response to inotropic support observed in current study.

A previous study (Snapir et al., 2003) has shown that systemic infusions of β AR agonist (epinephrine) are associated with larger vasodilation in subjects homozygous for the Arg16- β_2 AR compared to those carrying one or two Gly16 alleles [28]. Theoretically, this larger vasodilation could be related to lower MAP or SBP in Arg16Arg- β_2 AR homozygotes but our study failed to find any influence of the Arg16Gly- β_2 AR polymorphism on hemodynamic parameters (SBP, $p = 0.839$ and MAP, $p = 0.0.669$). It might be due to other compensatory cardiovascular reflexes which may have overshadowed the agonist activity of catecholamines.

Taken together, chronotropic and inotropic effects of epinephrine were similar across all the different genotypes studied. Findings of the current research and several previous studies have demonstrated variable results [14, 19]. The reasons for these differences are not clearly defined, but may be related to differences in genetic properties of various races.

Limitations

Sample size of this study was relatively limited. It must be recognized that the lack of impact of these polymorphisms on hemodynamic response of post-CABG surgery patients observed in the present study is only hypothesis-generating and further researches with a bigger sample size and different ethnic background are certainly needed to give more reliable data for general population of Iranian patients. Our study demonstrated that the numbers of study's male participants were almost double the number of female patients which indicate that these results may be generalizable and applicable to male patients. As of now, several functionally important SNPs in the human β_1 and β_2 AR coding region have been described. Hence, evaluation of the other SNPs, particularly in the β_2 AR coding region such as Gln27Glu and Thr164Ile polymorphisms would have provided additional important information.

Conclusion

To conclude, our results demonstrated that in patients undergoing CABG surgery with CPB who carrying one or two alleles of the Arg389- β_1 AR, the time of inotrope support to achieve positive inotropic response was significantly shortest than in Gly389- β_1 AR homozygotes. Furthermore, data from this study revealed that no significant association between patient's hemodynamic response to inotropic support after CABG surgery and three polymorphisms studied have been observed in Iranian population. However, to provide stronger evidence further genetic analysis with different and larger populations is required.

Abbreviations

AR

Adrenoceptor; CABG:Coronary artery bypass grafting; CPB:Cardiopulmonary bypass; SBP:Systolic blood pressure; DBP:Diastolic blood pressure; MAP:Mean arterial pressure; CVP:Central venous pressure; HR:Heart rate; ICU:Intensive care unit; EDTA:Ethylene diamine tetraacetic acid; PCR:Polymerase chain reaction; RFLP:Restriction fragment length polymorphism, BMI:Body mass index, LVEF:Left ventricular ejection fraction; Clcr:Creatinine clearance; MI:Myocardial infarction; COPD:Chronic obstructive pulmonary disease; ACE:Angiotensin-converting enzyme; ARB:Angiotensin receptor blocker; LMCA:Left main coronary artery; CCT:Cross-clamp time; CPBT:Cardiopulmonary bypass time; MV:Mechanical ventilation

Declarations

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Authors' contributions

GE designed the study and edited the manuscript. RG and VH aided in the recruitment of the patients. AHO, OA and RA performed the genotyping and RFLP-PCR experiments. MM analyzed the data. SE was involved in collecting clinical data and manuscript writing. All authors read and approved the contents of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the author for correspondence upon reasonable request.

Ethics approval and consent to participate

The study was approved by the ethics committee of Mazandaran University of Medical Sciences (approval code: IR.MAZUMS.REC.1397.1810). All participants provided written voluntary informed consent prior to the study.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

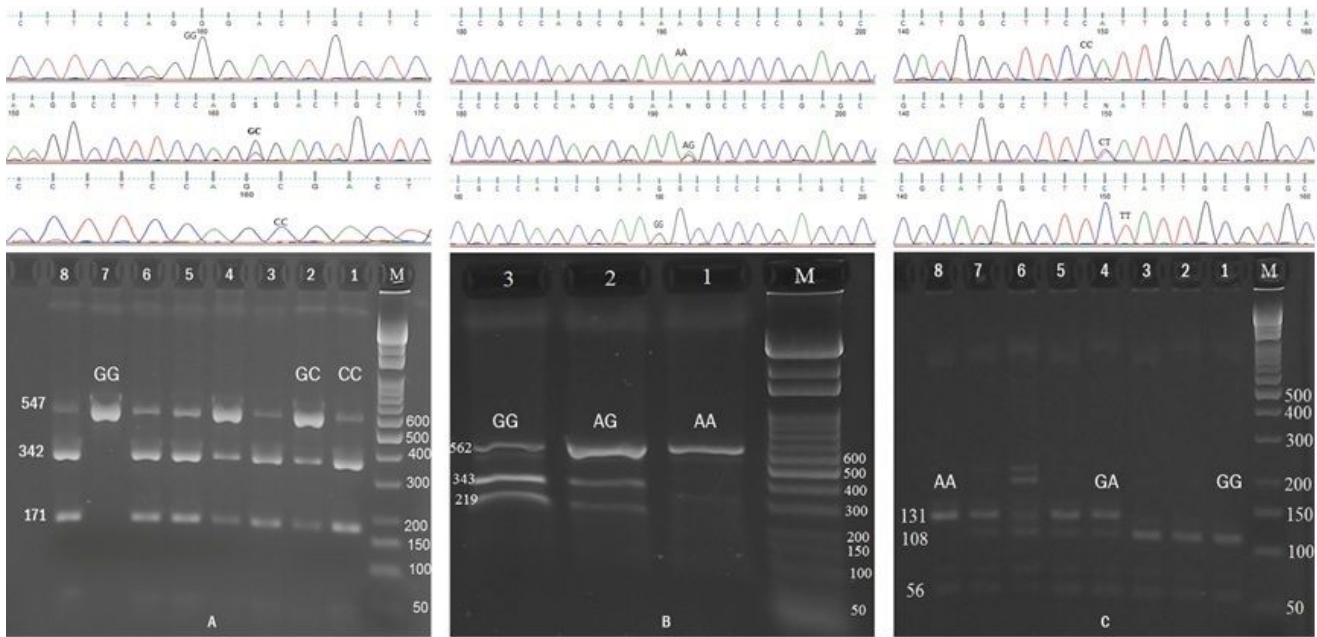


Figure 1

Sequence analysis and pattern of 2% agarose gel electrophoresis of DNA samples for Arg389Gly-β1AR (A), Ser49Gly-β1AR (B) and Arg16Gly-β2AR (C) polymorphisms detected by PCR-RFLP; Lane M shows 50bp DNA ladder; (A) the Arg389Gly GG genotype was evident as a single 547 bp fragment, GC genotype as 547,342, 171 and 34 bp fragments and CC genotype as 342, 171 and 34 bp fragments; (B) the Ser49Gly AA genotype was evident as a single 562 bp fragment, AG genotype as 562, 343 and 219 bp fragments and GG genotype as 343 and 219 bp fragments; (C) the Arg16Gly GG genotype was evident as 108, 56, 23 and 14 bp fragments, GA genotype as 131, 108, 56, 23 and 14 bp fragments and AA genotype as 131, 56 and 14 bp fragments (reverse direction); Electrofluorogram representing all heterozygous and homozygous condition of the β1 and β2-adrenoceptor alleles (Top).

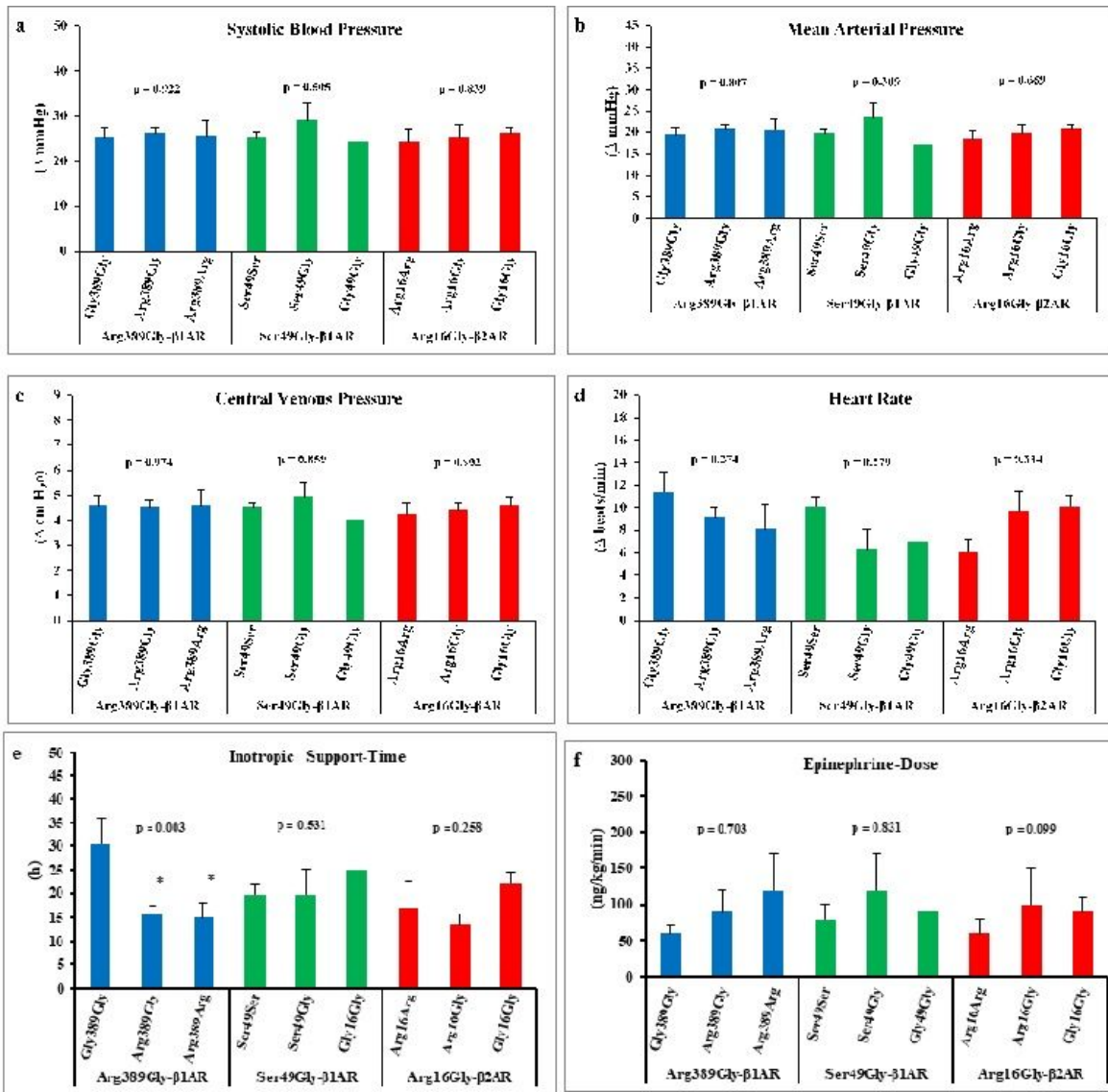


Figure 2

Effects of the Arg389Gly- β 1AR, Ser49Gly- β 1AR and Arg16Gly- β 2AR polymorphisms on the changes in hemodynamic parameters, dose and duration of inotropic support in post-CABG patients. [a. increase in systolic blood pressure (Δ mmHg), b. increase in mean arterial pressure (Δ mmHg), c. decrease in central venous pressure (Δ cm H₂O), d. increase in heart rate (Δ beats/min), e. inotropic support-time (h) and f. epinephrine-dose (ng/kg/min)]. Columns are mean and the vertical bars show the SEM. Significance is indicated by an asterisk (* p < 0.05 vs Gly389Gly).

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