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β_1-ADRENERGIC RECEPTOR POLYMORPHISMS AND RISK OF ATRIAL FIBRILLATION AFTER CARDIAC SURGERY IN IRANIAN POPULATION

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ABSTRACT: Postoperative atrial fibrillation (POAF) is a frequent rhythm disturbance after cardiac surgery and is associated with poor cardiovascular outcomes. Beta-blocker therapy is an effective strategy to prevent or decrease the high incidence of POAF and β_1-adrenergic receptor (β_1AR) polymorphisms that affect the cardiac response to β_1AR antagonists. The study objective is to analyze the association between genetic variations in the β_1AR gene and the risk of POAF and the prophylactic effect of beta-blockers. Totally, 198 patients undergoing first-time cardiac surgery were examined. The patients after operation were grouped as either with POAF or without POAF according to their continuously electrocardiogram monitoring within ICU and hospital admission period. Subsequently, genotype frequencies of the β_1AR gene were determined by polymerase chain reaction-restriction fragment length polymorphism. The results showed that 63 patients (31.8%) of the total study population developed POAF. After the application of a multivariate logistic regression model, the incidence of POAF was associated with Arg389Gly genotype (OR=2.48; 95% CI, 1.06-5.76; p=0.036), Arg389Arg genotype (OR=3.38; 95% CI, 1.28-9.33; p=0.018) and Arg389Ser49 haplotype (OR=1.73; 95% CI, 1.07-2.80; p=0.027) in the whole population. In addition, subgroup analysis showed an association between Arg389Gly (OR=3.09; 95% CI, 1.12-8.52; p=0.03) and Arg389Arg (OR=4.72; 95% CI, 1.07-15.43; p=0.01) genotypes as well as Arg389Ser49 haplotype (OR=2.05; 95% CI, 1.16-3.62; p=0.013) with increased risk for POAF in patients using beta-blocker prophylaxis. These findings indicated that POAF might be related to the Arg389-β_1AR variant in the studied Iranian population, and this association appears to be more prominent among patients with beta-blocker prophylaxis.

INTRODUCTION: Postoperative atrial fibrillation (POAF) occurs in 15-40% of patients in the first 24-48 postoperative hours after coronary artery bypass graft (CABG) and 62% undergoing valve procedure plus CABG, which is a leading cause of morbidity and mortality [1].

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Moreover, it increases the length of intensive care unit (ICU) stays or hospitalization and health care cost [2]. Currently, atrial fibrillation (AF) rhythm is the most common indication for readmission within 30 days after hospital discharge [3, 4].

In recent years, the developments in the field of pharmacotherapy and advances in genetic knowledge, including the discovery of novel atrial fibrillation-related genetic variants and extension to new insights into AF mechanisms, have led to patient-specific management and reduced the POAF complications, including the need for a pacemaker, heart failure, venous thromboem-

bolism, myocardial infarction and stroke [5, 6]. The exact pathophysiology of POAF remains unclear, and its causes are likely multifactorial [7]. Inflammation and structural atrial changes occur at the time of surgery and postoperative period, including trauma following cannulation, acute atrial dilatation, ischemic injury, and elevation in atrial pressures or hypertension predisposes patients to POAF [1, 8].

Evidence has revealed that an increase in sinus rate and atrial ectopic activity secondary to an increased sympathetic tone is associated with POAF [2]. Hence, beta-blockers have been used extensively for prophylaxis of AF after cardiac surgery, and the efficacy of them as first-line agents for the prevention of POAF has been confirmed in several clinical trials and meta-analysis [9, 10]. However, several studies have elucidated that differences in response to β_1-adrenergic receptor (β_1AR) antagonists could have been influenced by considerable interindividual and interethnic variations [11, 12]. These findings suggest that the genetic variation of the β_1AR gene may be the basis of interindividual differences in response to beta-blocking medications as prophylaxis for POAF in patients after cardiac surgery. The β_1AR is a member of cell surface G protein-coupled receptors expressed on cardio-myocytes. They mediate the increase in cardiac inotropy and chronotropy and modulate sympathetic responses, an increase in intracellular cyclic adenosine monophosphate (cAMP) activity. The β_1AR gene is encoded by an intronless gene located on the long arm of chromosome 10q24-26 [13].

Two common functional single nucleotide polymorphisms (SNPs) in the human β_1AR gene (Arg389Gly and Ser49Gly) have been proved to play a pivotal role in the regulation of the activity of the cardiovascular and sympathetic nervous system [14]. *In vitro* studies have confirmed that the Gly49-β_1AR allele enhanced agonist-promoted down-regulation compared to the Ser49 variant, whereas the Arg389-β_1AR allele has nearly 2-fold greater basal and 3-fold greater agonist stimulation of the adenylyl cyclase activity than the Gly389-β_1AR variant [15]. The Arg389-β_1AR variant displays an increase in Gs-protein coupling compared to Gly389-β_1AR [16]. Parvez *et al*., studied the effects of β_1AR gene polymorphisms (Arg389Gly and Ser49Gly) on rate-control therapy in patients with AF. They found that patients carrying Gly389-β_1AR variant were associated with an adequate response to rate-control therapy and required the lowest doses of rate-control medications than patients with the Arg389-β_1AR allele.

Moreover, they also found that the Ser49Gly polymorphism did not associate with the outcome of rate-control therapy in patients with AF [17]. Due to the prominent role of increased sympathetic tone in susceptibility to POAF and the effect of genetic variations of the β_1-adrenergic receptor as a mediator of cardiac sympathetic activation on response to beta-blocker therapy, we designed a prospective study to examine the possible association of the SNPs in the β_1AR gene with the risk for POAF and influence on the outcome of prophylactic beta-blocker therapy in Iranian patients after cardiac surgery.

MATERIALS AND METHODS:

Study Population: The study protocol was approved by the ethics committee of Mazandaran University of Medical Sciences (approval code: IR. MAZUMS.REC.1397.1810), and voluntary informed consent was obtained from enrolled participants for relevant clinical data gathering and genotyping analysis. Patients aged 18 years or older who indicated elective cardiac surgery were consecutively enrolled in the study between April and September 2018. The exclusion criteria were previous history of AF or other supraventricular and ventricular arrhythmias; those who also needed implanted cardiac devices, including a pacemaker or defibrillator, were excluded from the study. In addition, patients who had bradycardia (heart rate < 60 beats/min) and creatinine clearance < 30 ml/min were excluded. In total, 198 patients were recruited. The patients with an episode of postoperative atrial fibrillation that persisted for more than 15 minutes during the ICU and 5 days post-ICU were defined as POAF group (n=63), and subjects (n=135) without post-operatively AF episodes were classified as the without POAF (control group) [18]. In patients with AF rhythm, anti-coagulant agents and amiodarone or cardioversion were used to manage arrhythmia.

Definitions: POAF was defined according to the European Society of Cardiology (ESC) Guidelines

for the management of AF, based on no discernible P wave or irregular RR intervals or replacement of sinus P waves by rapid oscillations or fibrillatory waves that vary in size, shape, and timing which continued for at least 30 seconds [19]. Postoperative prophylactic beta-blocker treatment was defined as prescription of beta-blocker before the onset of AF rhythm in patients with POAF or during the ICU stay and up to 5 days after ICU discharge in subjects without POAF.

Clinical Data Collection: All the data related to before, during, and after the surgery, including demographic parameters, surgical information, medical and medication history of the patients were obtained from patient's medical records. All the patients were preoperatively assessed using electrocardiography (ECG) and transthoracic echo-cardiography examination. POAF was detected by continuous telemetry monitoring and a 12-lead ECG recording. Subsequently, all patients were examined at least twice a day by the surgeon and intensive care specialists in ICU and cardiologist on the post-ICU ward. During this time, any AF rhythm was confirmed and documented by the research team.

Anesthesia and Operative Techniques: Similar standard surgical technique with the cardio-pulmonary bypass (CPB) method was utilized in all the patients.

Genotyping: Five milliliters of peripheral venous blood specimens were obtained at the end of the operation for isolation of DNA. Total genomic DNA was extracted from the leukocytes using DNA isolation kit (Denazist Asia Co., Mashhad, Iran). The concentration and purity of the extracted DNA were measured using a Nanodrop spectro-photometer (Biochrom WPA Biowave II^{+} UV / visible spectrophotometer, Cambridge, UK) [20]. The Arg389Gly and Ser49Gly polymorphisms in the β_1AR were confirmed by polymerase chain reaction-restriction fragment length polymorphism (RFLP-PCR) methods.

The forward primer for the codon 389 (nucleotide 1165) was 5′-CATC ATGG GCGT CTTCA CGC-3′, and the reverse primer was 5′-T GGGC TTCG AGTT CACC TGC-3′. Primers used for codon 49 (nucleotide 145) had the following sequences: 5'-CCGG GCTT CTGG GGTG TTCC-3′ (forward), 5'-GGCG AGGT GATG GCGA GGTA GC-3' (reverse) [21].

PCR was performed using 200 ng of template DNA, 10 µl Taq DNA polymerase 2x master mix RED (Ampliqon A/S, Odense, Denmark) containing 150 mM Tris-HCl pH 8.5, 40 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM of each dNTP and 0.2 U/µl Ampliqon Taq DNA polymerase, along with 10 pmol of specific primers and 7 µl nuclease-free water.

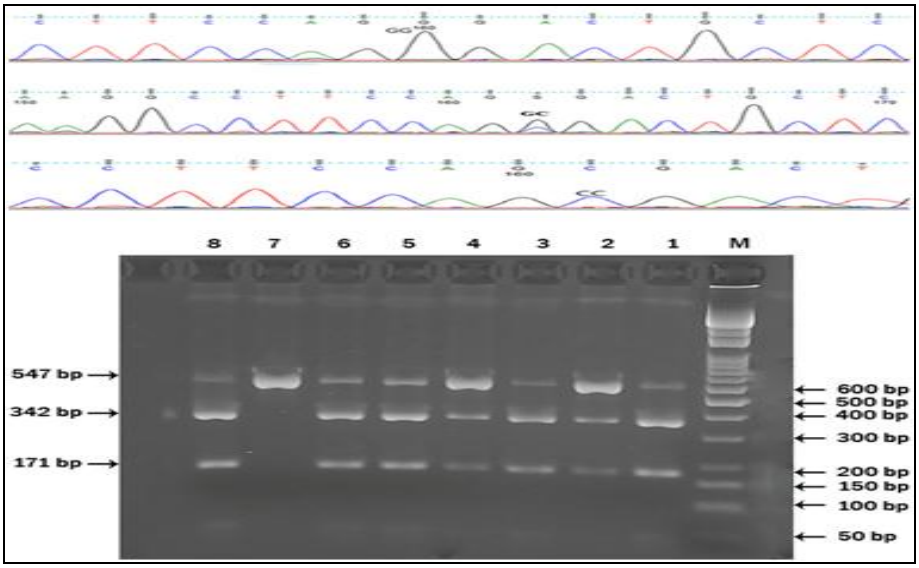

FIG. 1: SEQUENCE ANALYSIS AND PATTERN OF 2% AGAROSE GEL ELECTROPHORESIS OF DNA SAMPLES FOR ARG389GLY POLYMORPHISM DETECTED BY PCR-RFLP. Lane M shows a 50bp DNA ladder. The Arg389Gly GG genotype was evident as a single 547 bp fragment (Lane 7), GC genotype as 547, 342, 171, and 34 bp fragments (Lanes 2 and 4), and CC genotype as 342, 171, and 34 bp fragments (Lanes 1, 3, 5, 6 and 8). Electrofluorogram was representing all heterozygous and homozygous conditions of the β_1-adrenoceptor allele (top).

The PCR conditions were: initial denaturation step at 98 ºC for 3 min followed by 35 cycles of denaturation at 98 ºC for 30 sec, annealing at 60 ºC for 1 min and 72 ºC for 30 sec for β_1AR codons 389 and 49, following confirmation of the amplified fragments of the expected size on agarose gel containing DNA green fluorescent dye (DenaZist, Mashhad, Iran).

The expected product lengths were 547 bp and 562 bp for β_1AR codons 389 and 49, respectively. The PCR products were restriction-digested with 10 U/µl of *Bcg*I and *EcoO109*I (Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37 ºC for 3 h. According to the digestion patterns, two genotypes were determined **Fig. 1** and **Fig. 2**.

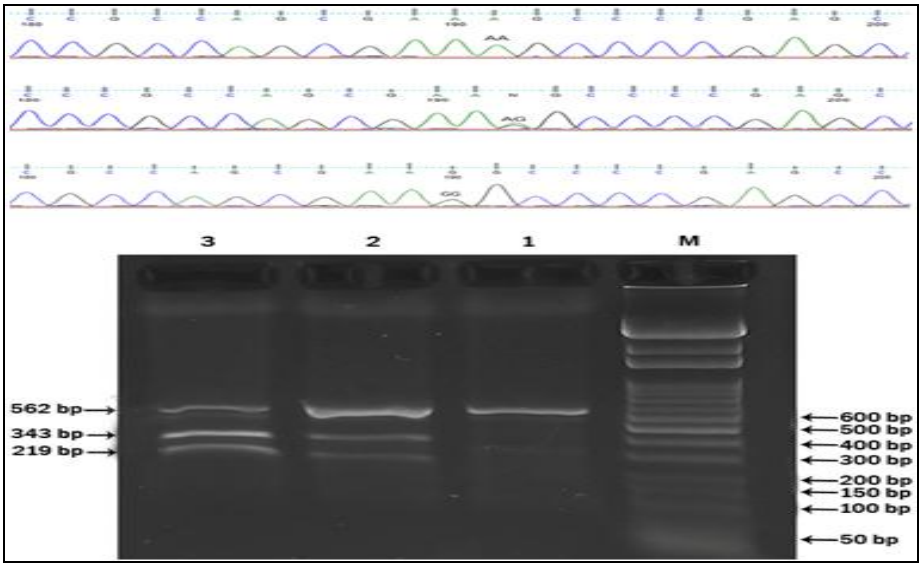

FIG. 2: SEQUENCE ANALYSIS AND PATTERN OF 2% AGAROSE GEL ELECTROPHORESIS OF DNA SAMPLES FOR SER49GLY POLYMORPHISM DETECTED BY PCR-RFLP. Lane M shows a 50bp DNA ladder. The Ser49Gly AA genotype was evident as a single 562 bp fragment (Lane 1), AG genotype as 562, 343, and 219 bp fragments (Lane 2) and GG genotype as 343 and 219 bp fragments (Lane 3). Electrofluorogram representing all heterozygous and homozygous conditions of the β1-adrenoceptor allele (top).

PCR Products Sequencing: PCR-RFLP genotyping results were further confirmed by direct DNA sequencing **Fig. 1** and **Fig. 2**. Genotyping was performed without knowing the population study status. Ten percent of random samples were re-genotyped by Sanger sequencing with forward and reverse primers by ABI 3130XL.

Statistical Analysis: All statistical analyses were carried out using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). All the statistical tests were two-sided, and a P-value of < 0.05 was considered statistically significant. Values are shown as range and mean ± standard deviation (SD). The Differences between means and categorical variables were tested by *t*-test and Chi-square analysis, respectively. The Chi-square test was used to analyze the differences in distributions of genotypes and alleles between patients with and without POAF. Logistic regression analysis was performed to assess the association of β_1AR genotypes with POAF and adjusted for the following clinical covariates: Age ≥ 65 years, male gender, BMI ≥ 30 (kg / m^2), reduced left ventricular ejection fraction (LVEF) state (< 50%), valvular surgery, postoperative left atrial diameter (LAD) ≥ 40 mm, cross-clamp time (CCT) and cardiopulmonary bypass time (CPBT). In addition, Multivariate logistic regression analysis was also performed with and without adjustment on the resulting data to determine the interaction between prophylactic beta-blocker use and β_1AR genotype on the development of POAF.

RESULTS: The incidence of POAF among our patients was 31.8%, and 155 (78.3%) of 198 patients received postoperative prophylactic beta-blocker therapy. The mean duration of POAF was 20.3 ± 18.1 h (range of 1 to 61 h). The highest prevalence of POAF was recorded on the second and third post-operative days. The results revealed no significant difference between the two groups regarding baseline characteristics **Table 1**.

The mean age of the study patients was 63.3 ± 9.3 years (range of 36 to 87 years), and 65.7% were men. CABG (87.9%) was the most frequent underlying heart procedure in the study population. As shown in **Table 1**, surgical and post-operative data were also similar, except for the operation time, which was significantly higher in patients with POAF than those without POAF (4.2 ± 0.9 h *vs* 3.9 ± 0.9 h, p=0.042).

TABLE 1: BASELINE AND OPERATIVE CHARACTERISTICS OF THE STUDY POPULATION

Characteristics	With POAF (n=63)	Without POAF (n=135)	P-Value
Age (year)	65.1 ± 9.9	62.6 ± 9	0.086
Male gender, n	40 (63.5%)	90 (66.7%)	0.661
BMI (kg/m^2)	27.1 ± 4.1	26.9 ± 4.2	0.855
EuroScore II	2.1 ± 1.5	1.9 ± 1.6	0.409
Smoking, n	15 (23.8%)	41 (30.4%)	0.340
Comorbid features, n			
Previous MI	8 (12.7%)	22 (13.6%)	0.511
Hypertension	43 (66.2%)	87 (64.4%)	0.599
Diabetes mellitus	22 (34.9%)	57 (42.2%)	0.328
COPD	1 (1.6%)	6 (4.4%)	0.434
NYHA functional class, n			0.807
I	8 (12.7%)	18 (13.3%)	
II	12 (19%)	21 (15.6%)	
III	2 (3.2%)	10 (7.4%)	
IV	1 (1.6%)	2 (1.5%)	
Medication status, n			
BB	54 (85.7%)	118 (87.4%)	0.742
ACEI	34 (54%)	61 (45.2%)	0.249
ARB	18 (28.6%)	45 (33.3%)	0.358
LVEF (%)	54 ± 7.5	53.3 ± 9.1	0.606
Clcr (ml/min/1.73 m^2)	71 ± 21.1	73.9 ± 20.8	0.359
Surgery type, n			0.847
CABG	52 (82.5%)	122 (90.4%)	
Valvular surgery	11 (17.5%)	13 (9.6%)	
MVR MV-repair	2 (3.2%)	1 (0.7%)	0.144
AVR	6 (9.5%)	8 (5.9%)	0.086
Operation time (h)	3 (4.8%)	4 (3%)	0.525
Inotropic support use (Epinephrine), n	4.2 ± 0.9	3.9 ± 0.9	0.042
Epinephrine - dose (ng/kg/min)	25 (39.7%)	46 (34.1%)	0.064
Total inotropic support - time, (h)	46.8 ± 23.8	39.8 ± 15.7	0.193
CCT (min)	16.8 ± 9.1	15.1 ± 8.9	0.428
CPBT (min)	66.4 ± 35.2	62.9 ± 31.8	0.365
Postoperative LAD (mm)	102.7 ± 50.3	95.8 ± 46.2	0.487
	43.4 ± 4.1	42.5 ± 4.1	0.132

BMI: Body mass index, MI: Myocardial infarction, COPD: Chronic obstructive pulmonary disease, NYHA: New York heart association, BB: Beta-blocker, ACEI: Angiotensin-converting enzyme inhibitor, ARB: Angiotensin receptor blocker, CABG: Coronary artery bypass graft, MVR: Mitral valve replacement, AVR: Aortic valve replacement, CCT: Cross-clamp time, CPBT: Cardiopulmonary bypass time, LVEF: Left ventricular ejection fraction, LAD: Left atrial diameter, CLcr: Creatinine clearance. Values are mean ± SD where appropriate.

Genotype Analysis: As can be seen from **Table 2**, the frequencies of alleles and genotypes of Arg389Gly and Ser49Gly polymorphism were similar in both groups, and there was no association between the two polymorphisms studied and the incidence of atrial fibrillation after cardiac surgery in the total population. Multivariate logistic regression analysis showed that heterozygous Arg389Gly variant (OR=2.48; 95% CI, 1.06-5.76; p=0.036), mutant Arg389Arg genotype (OR=3.38; 95% CI, 1.28-9.33; p=0.018) and Arg389-allele (OR=1.72; 95% CI, 1.09-2.72; p=0.02) was significantly associated with POAF compared to the reference group. Genotype analysis revealed that patients with prophylactic beta-blocker therapy and POAF had a higher frequency of mutant Arg389Arg genotype (OR=3.13; 95% CI, 1.11-8.81; p=0.031) compared to control subjects (29.2% *vs* 17.7%).

TABLE 2: DISTRIBUTION OF GENOTYPES AND ALLELE FREQUENCIES OF THE β_1-ADRENERGIC RECEPTOR POLYMORPHISMS AND ITS RELATIONSHIP WITH POAF IN THE WHOLE STUDY POPULATION AND SUBGROUPS SEPARATED BY PROPHYLACTIC BETA-BLOCKER THERAPY

Allelic Status	With POAF	Without POAF	Unadjusted OR (95% CI)	P-value	Adjusted* OR (95%CI)	P-Value
All patients	(n=63)	(n=135)				
Gly389Gly	11 (17.5%)	41 (30.4%)	1.0 (Ref.)		1.0 (Ref.)	
Arg389Gly	36 (57.1%)	69 (51.1%)	1.95 (0.89 - 4.23)	0.094	2.48 (1.06 - 5.76)	0.036
Arg389Arg	16 (25.4%)	25 (18.5%)	2.39 (0.96 - 5.95)	0.062	3.38 (1.28 - 9.33)	0.018
Allele Arg389 *vs* Gly389	58 (46%) / 68 (54%)	151 (55.9%) / 119 (44.1%)	1.48 (0.97 - 2.28)	0.067	1.72 (1.09 - 2.72)	0.02
Ser49Ser	53 (84.1%)	106 (78.5%)	1.0 (Ref.)		1.0 (Ref.)	
Ser49Gly	10 (15.9%)	28 (20.7%)	0.71 (0.32 - 1.58)	0.406	0.7 (0.30 - 1.65)	0.416
Gly49Gly	0%	1 (0.8%)	-	-	-	-
Allele Gly49 *vs* Ser49	116 (92.1%) / 10 (7.9%)	240 (88.9%) / 30 (11.1%)	0.69 (0.33 - 1.46)	0.331	0.7 (0.32 - 1.54)	0.374
Prophylactic BB use						
Yes	(n=48)	(n=107)				
Gly389Gly	8 (16.6%)	34 (31.8%)	1.0 (Ref.)		1.0 (Ref.)	
Arg389Gly	26 (54.2%)	54 (50.5%)	2.05 (0.83 - 5.04)	0.119	3.09 (1.12 - 8.52)	0.03
Arg389Arg	14 (29.2%)	19 (17.7%)	3.13 (1.11 - 8.81)	0.031	4.72 (1.45 - 15.43)	0.01
Allele Arg389 *vs* Gly389	42 (43.8%) / 54 (56.2%)	122 (57%) / 92 (43%)	1.71 (1.05 - 2.77)	0.031	2.03 (1.19 - 3.47)	0.009
Ser49Ser	39 (61.9%)	81 (60%)	1.0 (Ref.)		1.0 (Ref.)	
Ser49Gly	9 (14.3%)	25 (18.5%)	0.59 (0.06 - 6.28)	0.666	0.71 (0.28 - 1.78)	0.465
Gly49Gly	0	1 (0.8%)	-	-	-	-
Allele Gly49 *vs* Ser49	87 (90.6%) / 9 (9.4%)	187 (87.4%) / 27 (12.6%)	0.72 (0.32 - 1.59)	0.412	0.71 (0.31 - 1.66)	0.427
No	(n=15)	(n=28)				
Gly389Gly	3 (20%)	7 (25%)	1.0 (Ref.)		1.0 (Ref.)	
Arg389Gly	10 (66.7%)	15 (53.6%)	1.56 (0.32 - 7.49)	0.582	1.69 (0.28 - 10.21)	0.568
Arg389Arg	2 (13.3%)	6 (21.4%)	0.78 (0.09 - 6.32)	0.814	1.01 (0.09 - 11.07)	0.992
Allele Arg389 *vs* Gly389	16 (53.3%) / 14 (46.7%)	29 (51.8%) / 27 (48.2%)	0.94 (0.39 - 2.29)	0.891	1.07 (0.38 - 2.98)	0.904
Ser49Ser	14 (93.3%)	25 (89.3%)	1.0 (Ref.)		1.0 (Ref.)	
Ser49Gly	1 (6.7%)	3 (10.7%)	0.75 (0.32 - 1.75)	0.504	0.22 (0.01 - 13.82)	0.473
Gly49Gly	0	0	-	-	-	-
Allele Gly49 *vs* Ser49	29 (96.7%) / 1 (3.3%)	53 (94.6%) / 3 (5.4%)	0.61 (0.06 - 6.12)	0.674	0.24 (0.01 - 12.52)	0.476

Gly: Glycine, Arg: Arginine, Ser: Serine, BB: Beta-blocker, Ref.: Reference category; OR: Odds ratio, CI: Confidence intervals. *Adjusted for age ≥ 65 years, male gender, BMI ≥ 30 (kg/m^2), reduced left ventricular ejection fraction (LVEF) state (< 50%), valvular surgery, postoperative left atrial diameter ≥ 40 mm, cross-clamp time (CCT) and cardiopulmonary bypass time (CPBT).

Additionally, the presence of the Arg389-allele was associated with POAF in patients received prophylactic beta-blocker therapy (OR=1.71; 95% CI, 1.05-2.77; p=0.031). Multivariate logistic regression analysis stratified by prophylactic beta-blocker therapy showed a significant association between Arg389-allele (OR=2.03; 95% CI, 1.19-3.47; p=0.009), Arg389Gly (OR=3.09; 95% CI, 1.12-8.52; p=0.03) and Arg389Arg (OR=4.72; 95% CI, 1.45-15.43; p=0.01) genotypes with the risk of POAF after adjustment for clinical covariates **Table 2**. According to the statistical analysis, in our patients stratified by prophylactic beta-blocker therapy, no influence of the Ser49Gly polymorphism on the incidence of POAF was found before and after adjustment for clinical previously described covariates.

TABLE 3: HAPLOTYPE ANALYSIS OF THE β_1-ADRENERGIC RECEPTOR POLYMORPHISMS AND ITS RELATIONSHIP WITH POAF IN THE WHOLE STUDY POPULATION AND SUBGROUPS SEPARATED BY PROPHYLACTIC BETA-BLOCKER THERAPY

	Haplotypes	With POAF	Without POAF	Unadjusted OR (95% CI)	P-value	Adjusted* OR (95% CI)	P-value
		(n=63)	(n=135)				
All patients	Gly389Ser49	28 (44.4%)	71 (52.6%)	1.0 (Ref.)			
	Arg389Ser49	30 (47.6%)	49 (36.3%)	1.50 (0.96 - 2.34)	0.075	1.73 (1.07 - 2.80)	0.027
	Gly389Gly49	1 (1.6%)	4 (3%)	0.28 (0.03 - 2.24)	0.228	0.22 (0.03 - 1.89)	0.168
	Arg389Gly49	4 (6.4%)	11 (8.1%)	1.07 (0.46 - 2.47)	0.878	1.28 (0.52 - 3.15)	0.586
Prophylactic BB use							
Yes		(n=48)	(n=107)	1.0 (Ref.)			

	Gly389Ser49	20 (31.7%)	57 (42.2%)	1.75 (1.05 - 2.93)	0.032	2.05 (1.16 - 3.62)	0.013
	Arg389Ser49	23 (36.5%)	37 (27.4%)	0.35 (0.04 - 2.87)	0.326	0.25 (0.03 - 2.22)	0.214
	Gly389Gly49	1 (1.6%)	4 (3%)	1.17 (0.48 - 2.88)	0.731	1.41 (0.54 - 3.69)	0.490
	Arg389Gly49	4 (6.4%)	9 (6.7%)				
No		(n=15)	(n=28)				
	Gly389Ser49	8 (12.7%)	14 (10.4%)	1.0 (Ref.)			
	Arg389Ser49	6 (9.5%)	12 (8.9%)	0.91 (0.37 - 2.26)	0.839	1.06 (0.38 - 2.98)	0.912
	Gly389Gly49	0	1 (0.7%)	-	-	-	-
	Arg389Gly49	d1 (1.6%)	1 (1.5%)	0.88 (0.07 - 10.43)	0.916	0.41 (0.01 - 27.73)	0.715

Gly: Glycine, Arg: Arginine, Ser: Serine, BB: Beta-blocker, Ref.: Reference category, OR: Odds ratio, CI: Confidence intervals. *Adjusted for age ≥ 65 years, male gender, BMI ≥ 30 (kg/m^2), reduced left ventricular ejection fraction (LVEF) state (< 50%), valvular surgery, postoperative left atrial diameter ≥ 40 mm, cross-clamp time (CCT), and cardiopulmonary bypass time (CPBT).

Haplotype Analysis: Haplotype analysis revealed that patients with Arg389Ser49 haplotype had 1.73 fold greater risk of POAF (OR=1.73; 95% CI, 1.07-2.80; p=0.027) compared to patients with the Gly389Ser49 reference haplotype **Table 3**. In patients receiving beta-blocker prophylaxis, the association between Arg389Ser49 haplotype and POAF was statistically significant in the unadjusted (OR=1.75; 95% CI, 1.05-2.93; p=0.027) or adjusted (OR=2.05; 95% CI, 1.16-3.62; p=0.013) models **Table 3**.

DISCUSSION: β_1-adrenergic receptor gene polymorphisms are involved pathophysiologically in cardiovascular diseases. In recent years an increasing number of studies on possible genetic associations with Arg389Gly-β_1AR and Ser49Gly-β_1AR polymorphisms on hypertension, heart failure, myocardial infarction, and left ventricular remodeling in response to beta-blockade have been reported [15].

The present study found that the Arg389Gly and Arg389Arg genotypes were associated with increased risk of POAF (OR=2.48; p=0.036 and OR=3.38; p=0.018, respectively). A similar association of Arg389Gly (OR=3.09; p=0.03) and Arg389Arg (OR=4.72; p=0.01) variants with POAF was observed in patients with prophylactic beta-blocker therapy. Our results also show that patients who carried Arg389Ser49 haplotype had increased risk for POAF compared to other haplotypes (OR=1.73; p=0.027). Furthermore, the incidence of POAF in subjects with prophylactic beta-blocker was associated with carrying the Arg389Ser49 haplotype (OR=1.75; p=0.032) it was more pronounced after adjustment for previously described clinical covariates (OR=2.05; p=0.013). The Arg389-β_1AR variant has a higher basal adenylyl cyclase activity than the Gly389-β_1AR variant that was due to reduced G-protein coupling for the Gly389-β_1AR [22]. *In-vitro,* isoprenaline-induced adenylyl cyclase activation was three- to fourfold higher in Arg389-β_1AR than in Gly389-β_1AR variant [23].

Additionally, the Gly49 mutant has been shown to be associated with down-regulated by long-term agonist exposure to a significantly greater extent than the Ser49 mutant [24]. Elevated post-operative plasma level of norepinephrine is a well-known risk factor for POAF [25]. Sandilands *et al*., in a study on human right atrial preparations, reported greater inotropic and cAMP responses to norepinephrine at the Arg389-β_1AR versus the Gly389-β_1AR variant [26]. Taken together, our findings and these data indicate that the patients with Arg389-β_1AR experienced POAF more frequently than others. Inflammation response and cardiomyocyte damage following cardiac surgery with CPB will lead to an increase in catecholamine release, which has been associated with an increase in stimulation of the β-adrenergic receptors. This results in increased intracellular concentrations of cAMP [27, 28]. Iso-prenaline-evoked cAMP levels were significantly higher in Arg389Gly49 and Arg389Ser49 haplo-types than Gly389Ser49 haplotype.

Additionally, recent study reported that the patients with Arg389Gly49, Arg389Ser49, and Gly389Ser 49 haplotypes were more susceptible to idiopathic ventricular arrhythmias [28]. As shown in our study patients, the Arg389Ser49 haplotype of the β_1AR is associated with increased susceptibility to the provocation of POAF than other haplotypes. This was maybe due to better coupling of the Arg389Ser49 haplotype to the Gs-protein and inducing higher intracellular cAMP levels [29].

Clinical studies examining the influence of β_1AR polymorphism on beta-blocker response in patients with AF have met with variable results. Previous studies found a lower rate of new-onset AF in patients with heart failure and treated with bucindolol carrying the Arg389Arg genotype. Furthermore, this treatment effect was not observed in the patients with Gly389Gly variant [30-32]. However, in a study by VaglioJr *et al*., no significant difference between Arg389Gly-β_1AR and Ser49Gly-β_1AR polymorphisms and modulate response to rate-control therapy (beta-blocker) in AF patients was observed, whereas in other study, Gly389-β_1AR was shown to be associated with an adequate response to rate-control therapy in atrial arrhythmias [17, 33].

In our study, when wild-type homozygote genotype (Gly389Gly) was considered as reference, the association between Arg389Gly-β_1AR polymorphism and POAF was statistically significant for Arg-β_1AR variant among patients receiving prophylactic beta-blocker therapy. It seems that the higher risk of POAF in subjects with the Arg-β_1AR variant did not reduce by prophylactic treatment with beta-blockers. However, further studies are needed to validate our findings in the Iranian population. Our results show that Ser49Gly-β_1AR poly-morphism was not associated with the development of AF after cardiac surgery and also did not influence the outcome of prophylactic beta-blocker therapy in patients with POAF. However, our results differ from those of Nia *et al*., who suggested that the Arg389Gly genotype alone was not, and the Ser49Gly genotype slightly associated with the AF prevalence [23]. Interestingly, they reported an almost 7-fold higher risk for AF in a combination of both SNPs (Arg389Gly-Ser49Gly). Ethnic differences between the studied populations, sampling, and method differences may account for some of this discrepancy.

The major limitation of our study was the relatively small sample size. In addition, we have genotyped patients only for the β_1AR gene polymorphisms and not for the β_2AR gene variants. Genetic variation in the β_2AR gene might be associated with an increase in susceptibility to POAF because the previous evidence showed that the identification of β_2AR gene polymorphisms could be helpful for the prediction of ventricular arrhythmias [28].

CONCLUSION: We have shown that the carriage of the Arg389-β_1AR variant as a predictive factor might confer an elevated risk of developing POAF in the Iranian population. The results of this study also suggest that the protective effect of beta-blocker therapy may have no or less influence on the prevention of POAF in patients with the Arg389-β_1AR variant. This was the first study in this field conducted on POAF patients in Iran. Further confirmatory studies are needed to determine the clinical significance of these findings.

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