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Research Article

Beta$_2$-Adrenergic Receptor Gene Polymorphisms in Egyptian Patients with Acute Myocardial Infarction

Eman Toraih,[1] Mohammad H. Hussein,[2] and Dahlia I. Badran[3]

[1]*Genetics Unit, Department of Histology and Cell Biology, Faculty of Medicine, Suez Canal University, Ismailia 41522, Egypt*
[2]*Department of Chest Diseases, Faculty of Medicine, Cairo University, Giza, Egypt*
[3]*Department of Medical Biochemistry, Faculty of Medicine, Suez Canal University, Ismailia 41522, Egypt*

Correspondence should be addressed to Eman Toraih; emantoraih@gmail.com

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Background. Beta$_2$-adrenergic receptor (*ADRB2*) gene polymorphisms, Arg16Gly and Gln27Glu, have been implicated in the pathogenesis of cardiovascular diseases. The aim of this study was to determine the association of these two polymorphisms with the risk of myocardial infarction (MI) in the Egyptian population. *Methods*. Blood samples were collected from 68 MI patients and 75 healthy controls. They were assessed for the presence of cardiovascular risk factors and genotyped for the Arg16Gly (*rs1042713*) and Gln27Glu (*rs1042714*) polymorphisms using allelic-discrimination polymerase chain reaction. *Results*. There is no significant difference in genotype and allele frequencies at codon 16 between MI patients and controls ($P = 0.919$). However, at codon 27, MI risk was higher in Gln_{27} homozygous participants than in Glu_{27} carriers ($P = 0.045$). The haplotype frequency distribution showed significant difference among cases and controls ($P = 0.002$); homozygotes for Gly_{16}/Gln_{27} haplotype were more susceptible to MI than Gly_{16}/Glu_{27} carriers. Patients with Arg_{16}/Gln_{27} haplotype had higher serum total cholesterol levels ($P < 0.05$) and lower frequency of diabetes in MI patients ($P < 0.01$). However, both Glu_{27} genotypes and haplotype showed lower frequency of hypertension ($P < 0.001$). *Conclusions*. Our findings suggested that the *ADRB2* gene polymorphisms may play an important role in susceptibility of MI among Egyptian population.

1. Introduction

Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide, accounting for up to 40% of all deaths [1, 2]. It is caused by myocardial cell death due to prolonged ischemia [3]. MI is a multifactorial, polygenic disorder driven by interactions of an individual's genetic background and several environmental factors [4–7]. Greater understanding of MI etiology is mandatory to identify individuals at high risk and to improve prevention and therapy of this common and important condition. Cardiovascular risk factors, as smoking, obesity, hypertension, dyslipidemia, and diabetes, have long been known [8]. However these risk factors do not explain why some individuals are more susceptible to these environmental determinants in comparison to others with the same given risk factors. Several genomic association studies, family-based and twin studies, and linkage analysis provide insights into multiple susceptible genes underlying MI disease [9, 10].

The beta 2-adrenergic receptors (β_2-AR), a member of the G-protein-coupled receptor superfamily, have attracted great attention due to their multiple physiological and health effects, particularly those involving cardiovascular phenotypes [11–16]. In vascular smooth muscle, β_2-AR mediate vasodilation in response to adrenergic agonists, and, in healthy myocardium, they mediate chronotropic and inotropic responses to endogenous and exogenous adrenergic agents [17–21]. These functions render β_2-AR an important target for cardiovascular disease (CVD) therapy [15, 22, 23]. Interestingly, studies reported β_2-AR dysfunction to be implicated in the pathogenesis of several CVD including hypertension [24–27], coronary heart disease [13, 23], idiopathic dilated cardiomyopathy [28], congestive heart failure [29, 30], idiopathic ventricular arrhythmias [31], and sudden cardiac death [32].

Several single nucleotide polymorphisms (SNP) in the β_2-AR (*ADRB2*) gene (MIM# 109690) have been identified in

many populations [33, 34]. The most important two SNPs are located within the amino-terminal extracellular domain near the receptor's ligand-binding site [35]. The first SNP was caused by a change of adenine to guanine (A > G) at nucleotide 46 resulting in amino acid substitution from arginine to glycine at codon 16 (Arg16Gly or R16G). The second SNP is the change of cytosine to guanine (C > G) at nucleotide 79 causing the substitution from glutamine to glutamic acid at codon 27 (Gln27Glu or Q27E) [15, 36, 37]. *In vivo* and *in vitro* functional studies suggested that these two polymorphisms have altered receptor function and behavior [38–42]. Several clinical and pharmacological studies highlighted the important role of these polymorphisms in the pathogenesis of CVD and their impact on drug response [22, 30, 39, 43]. However, evidence regarding the role of *ADRB2* gene polymorphisms in patients with MI is ambiguous and inconsistent. Significant differences in the genotype frequencies and haplotype structure of *ADRB2* gene were noted in different ethnic populations [13, 23, 25, 44, 45]. Thus, this case-control study was conducted to determine the prevalence of these *ADRB2* gene polymorphisms in Egyptian population and to assess their genetic association with the susceptibility to MI disease.

2. Materials and Methods

2.1. Study Participants. The study population was composed of 68 patients with acute myocardial infarction (MI) and 75 age-matched healthy controls with no history of cardiovascular events, hypertension, or any other chronic diseases. Patients were recruited from Coronary Care Unit (CCU), Suez Canal University Hospital, Ismailia, Egypt, during the period between April 2013 and March 2014. The diagnosis of MI was confirmed by evidence of symptoms in the presence of either diagnostic elevations of cardiac enzymes or diagnostic changes on electrocardiograms (ECG) [46]. The cardiovascular risk factors were assessed in all participants. They underwent evaluations including measurement of blood pressure, body mass index (BMI), fasting serum lipid and glucose levels, cardiac enzymes, and ECG. Diabetic patients either had a known history of diabetes mellitus or were diagnosed according to the American Diabetic Association recommendations [47]. Hypertension was defined as the average SBP ≥ 140 mmHg and/or the average DBP ≥ 90 mmHg and/or self-reported current treatment for hypertension with antihypertensive medication [48]. The study was approved by the Medical Research Ethics Committee of Faculty of Medicine, Suez Canal University. Written informed consent was obtained from all participants.

2.2. Samples Collection and Biochemical Measurements. Fasting blood samples were collected from both cases and controls. Blood from patients was collected within 24 hours of CCU admission. Two milliliters (mL) of venous blood was withdrawn in EDTA tubes to be used for subsequent genetic analysis. Another two milliliters was collected in Vacutainer Serum Separator Tubes II (*Becton Dickinson Plymouth*) to obtain serum. Parameters such as fasting blood sugar (FBS), total cholesterol (TC), triglycerides (TG), high density lipoproteins-C (HDL-C), and low density lipoprotein-C (LDL-C) were analyzed within 2 h after collection. Fasting blood sugar was estimated using Roche/Hitachi analyzer kits based on the glucose oxidase and peroxidase method [49]. Serum TC and TG were measured by enzymatic colorimetric determination method using Roche/Hitachi cholesterol kit (Cat. number 1489232) [50, 51] and Roche/Hitachi triglycerides kit (Cat. number 1488872) [52]. HDL-C was measured using Roche/Hitachi HDL-C kit (Cat. number 03030024) and was estimated by phosphotungstic acid precipitation followed by enzymatic analysis in supernatant fraction [53]. LDL-C was calculated by the Friedewald formula after considering its limitations if TG > 400 mg/dL: LDL-C = TC − (HDL-C + TG/5) [54]. FBS < 126 mg/dL, TC levels <200 mg/dL, TG levels <150 mg/dL, LDL-C < 130 mg/dL, and HDL-C > 60 mg/dL were considered normal [55].

2.3. Genotyping. Genomic DNA was extracted from blood leukocytes using QIAamp DNA Blood Mini kit (QIAgen, Clinilab Co., Egypt, Catalog number 51104) according to the manufacturer's instructions [56]. Concentration and purity of the extracted DNA were measured by NanoDrop ND-1000 (NanoDrop Tech., Inc. Wilmington, DE, USA). Genotyping of the *ADRB2* polymorphisms (Arg16Gly, *rs1042713,* and Gln27Glu, *rs1042714*) was performed by real-time polymerase chain reaction (RT-PCR) technology. PCR was performed in a 25-μL reaction volume containing 12.5 μL Taqman Universal PCR Master Mix, No AmpErase UNG (2x), 20 ng genomic DNA diluted to 11.25 μL with DNase-RNase-free water, and 1.25 μL 20 × TaqMan SNP Genotyping Assay Mix (Applied Biosystems, Egypt, assay ID C_2084764_20 and C_2084765_20). Internal positive controls and no-template controls (no DNA) were included in each run. PCR amplification was carried out in an AB 7500HT instrument with the Sequence Detection System (SDS) Software version 2.1.1 (Applied Biosystems, Egypt) according to the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 92°C for 15 sec and 60°C for 1 min. Genotyping was performed blinded to case/control status. Ten per cent of the randomly selected samples were regenotyped in separate runs to exclude the possibility of false genotype calls with 100% concordance rate.

2.4. Statistical Analysis. Statistical analysis was carried out using the Microsoft Excel 2010 and the *"Statistical Package for the Social Sciences (SPSS) for windows"* software, version 20. Odds ratios (OR) with a 95% confidence interval (CI) were calculated. Categorical variables were compared using the chi-square (χ^2), while Mann-Whitney U and Kruskal-Wallis tests were used to compare continuous variables. Due to significant differences in some cardiovascular risk factor variables between cases and control participants, binary logistic regression analysis was performed that included the *ADRB2* polymorphism and potential confounders (age, gender, smoking status, family history of cardiovascular disease, prevalence of obesity, and hyperlipidemia). A two-tailed P value of 0.05 was considered statistically significant. The allele frequency within

each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. The carriage rate was calculated as the number of individuals carrying at least one of the investigated alleles divided by the total number of individuals in each group [57, 58]. The Hardy-Weinberg equilibrium was calculated using the Online Encyclopedia for Genetic Epidemiology (OEGE) software (http://www.oege.org/software/hwe-mr-calc.shtml) and tested by χ^2 test to compare the expected genotype frequencies among patient and control groups.

3. Results

3.1. Molecular Analysis of ADRB2 Gene Polymorphisms. A total of 68 patients and 75 controls were included in the analysis. Characteristics of the study population are shown in Table 1. SNP analysis of Arg16Gly (R16G; *rs1042713*) and Gln27Glu (Q27E; *rs1042714*) polymorphisms of *ADRB2* gene showed that the frequencies of Arg_{16} and Gly_{16} alleles in our study population were 0.37 and 0.63, while those of Gln_{27} and Glu_{27} alleles were 0.67 and 0.33, respectively. The distribution of Arg16Gly genotypes and alleles did not differ significantly between MI patients and controls. For Gln27Glu polymorphism, Gln_{27}/Glu_{27} was significantly the most prevalent genotype in the healthy control group (50.7%) while Gln_{27}/Gln_{27} was the most predominant genotype among patients (50%) ($P = 0.045$). The frequency of Glu_{27} allele was significantly lower in MI patients compared to controls (33.1% *versus* 45.3%; $P = 0.034$); see Table 2. The distribution of *ADRB2* genotypes among patients and controls was found in accordance with those expected by the Hardy-Weinberg equilibrium ($P > 0.05$). Haplotype analysis revealed the presence of three haplotypes only: Arg_{16}/Gln_{27}, Gly_{16}/Gln_{27}, and Gly_{16}/Glu_{27}. Their frequencies were 0.30, 0.29, and 0.41, respectively. Different combinations of haplotypes existed in the study population and showed significant difference between MI patients and healthy controls ($P = 0.002$); see Table 3. The frequency of Gly_{16}/Glu_{27} haplotype was significantly lower in MI patients than controls ($P = 0.045$). In addition, homozygote individuals for Gly_{16}/Gln_{27} haplotype had higher risk of developing MI compared to noncarriers, with adjusted OR (95% CI) of 20.4 (1.4–296); see Table 4.

3.2. ADRB2 Gene Polymorphisms and Disease Characteristics. Characteristics of patients according to *ADRB2* genotypes and haplotypes are demonstrated in Tables 5 and 6. Diabetes mellitus (DM) frequency was significantly higher in Gly_{16}/Gly_{16} genotype carriers (62.5%) compared to other genotypes carriers (6.2% and 31.3% for Arg_{16}/Arg_{16} and Arg_{16}/Gly_{16}, resp.) ($P = 0.003$). The frequency of hypertension was significantly higher in MI patients with Gln_{27}/Gln_{27} genotype (66.7%) compared to their counterparts (25% for Gln_{27}/Glu_{27} and 8.3% for Glu_{27}/Glu_{27}) ($P < 0.001$). In addition, MI patients with the Arg_{16}/Gln_{27} haplotype had significantly higher serum cholesterol levels compared to noncarriers (206 ± 36 *versus* 188 ± 42, $P = 0.03$).

Table 1: Baseline characteristics of the study groups.

Variables	Patients ($n = 68$)	Controls ($n = 75$)	P value*
Risk factors for CVD			
Age, y	53.5 ± 7.4	52.8 ± 6.1	*0.203*
Male gender, %	45 (66.2)	53 (70.7)	*0.252*
Smokers, %	37 (54.4)	15 (20.0)	***<0.001***
FH of CVD, %	35 (51.5)	17 (22.7)	***<0.001***
BMI, kg/m^2	29.5 ± 3.6	27.4 ± 2.7	***<0.001***
Hypertension, %	48 (70.6)	—	
SBP, mmHg	129.7 ± 22.7	122.0 ± 11.2	***0.010***
DBP, mmHg	83.8 ± 13.3	77.5 ± 7.7	***0.001***
Diabetic, %	32 (47.1)	—	
FBS, mg/dL	129.2 ± 45.7	98.2 ± 11.3	***<0.001***
Lipid profile			
Hyperlipidemia, %	62 (91.2)	38 (50.6)	***<0.001***
TC, mg/dL	199 ± 40	174 ± 40	***0.002***
TG, mg/dL	203 ± 63	151 ± 84	***<0.001***
HDL-C, mg/dL	38 ± 7.4	47.4 ± 12	***<0.001***
LDL-C, mg/dL	121 ± 41	96 ± 40	***0.001***
VLDL-C, mg/dL	40.5 ± 13	30.2 ± 17	***<0.001***
Cardiac enzymes			
CK, ng/mL	1688 ± 1832	—	
CK-MB. ng/mL	320 ± 370	—	
LDH, IU/L	1562 ± 1223	—	

Values are presented as number (percentage) or mean ± standard deviation. Chi-square (χ^2) and Mann-Whitney U tests were used. *Statistically significant at $P < 0.05$; FH: family history; CVS: cardiovascular disease; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; TC: total cholesterol; TG: triglyceride; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; CK: total creatine kinase; CK-MB: creatine kinase isoenzyme MB fraction; LDH: lactate dehydrogenase.

4. Discussion

To the best of our knowledge, this is the first work examining the association of Arg16Gly and Gln27Glu polymorphisms of beta$_2$-adrenergic receptor with acute myocardial infarction in Egyptian population. Frequency distribution of alleles and genotypes was in Hardy-Weinberg equilibrium for both SNPs. Gly_{16} and Gln_{27} were the most prominent alleles, representing 0.63 and 0.67 of the population, respectively. Correspondingly, Gly_{16}/Gly_{16} and Arg_{16}/Gly_{16} at codon 16 and Gln_{27}/Gln_{27} and Gln_{27}/Glu_{27} at codon 27 were the most common genotypes in the Egyptian population. This was in agreement with another Egyptian study where Gly_{16} and Gln_{27} were the most frequent alleles representing 0.60 and 0.75, respectively [59]. Similar frequencies in prior studies were found in different ethnic populations (African American, Caucasian, and Saudian populations) [33, 35, 39, 60, 61]. In contrast, individuals with Asian descent reported higher frequencies of Arg_{16} and Gln_{27} alleles and absence of Glu_{27}/Glu_{27} genotype [39, 59, 60, 62–64]. Haplotype analysis revealed the presence of 3 haplotypes only; their frequencies were 30% for Arg_{16}/Gln_{27}, 29% for Gly_{16}/Gln_{27}, and 41%

Table 2: Genotype and allele frequencies of *ADRB2* gene polymorphisms in patients and controls.

Variables	Patients ($n = 68$)	Controls ($n = 75$)	χ^2	P value	Crude OR (95% CI)	Adjusted[&] OR (95% CI)
Arg16Gly polymorphism						
Genotype frequency						
Arg_{16}/Arg_{16}	10 (14.7)	08 (10.7)	0.010	0.919	1.0	1.0
Arg_{16}/Gly_{16}	30 (44.1)	40 (53.3)			0.6 (0.2–1.7)	0.3 (0.07–2.0)
Gly_{16}/Gly_{16}	28 (41.2)	27 (36.0)			0.8 (0.2–2.4)	1.9 (0.3–9.9)
Allele frequency						
Arg_{16} allele	50 (36.8)	56 (37.3)	0.009	0.920	1.0	0.2 (0.1–0.7)
Gly_{16} allele	86 (63.2)	94 (62.7)			1.0 (0.6–1.6)	0.8 (0.2–3.7)
Gln27Glu polymorphism						
Genotype frequency						
Gln_{27}/Gln_{27}	34 (50.0)	22 (29.3)	4.012	**0.045**[*]	1.0	1.0
Gln_{27}/Glu_{27}	23 (33.8)	38 (50.7)			**0.3 (0.1–0.8)**[*]	0.5 (0.1–1.5)
Glu_{27}/Glu_{27}	11 (16.2)	15 (20.0)			0.4 (0.1–1.2)	1.9 (0.4–8.2)
Allele frequency						
Gln_{27} allele	91 (66.9)	82 (54.7)	4.459	**0.034**[*]	1.0	0.2 (0.06–1.1)
Glu_{27} allele	45 (33.1)	68 (45.3)			**0.5 (0.3–0.9)**[*]	0.5 (0.1–1.5)

Values are shown as number (%). Chi-square (χ^2) for trend was used. OR (95% CI): odds ratio and confidence interval. [&]Adjusted for confounding factors. Adjusted OR for alleles was calculated as presence versus absence of this particular allele. [*]Statistically significant at $P < 0.05$.

Table 3: Haplotype combinations of *ADRB2* gene polymorphisms in patients and controls.

Haplotype combinations	Patients ($n = 68$)	Controls ($n = 75$)	χ^2	P value	Crude OR (95% CI)	Adjusted[&] OR (95% CI)
Gly_{16}/Glu_{27} + Gly_{16}/Glu_{27}	11 (16.2)	15 (20.0)	9.404	**0.002**[*]	1.0	1.0
Gly_{16}/Glu_{27} + Gly_{16}/Gln_{27}	5 (7.40)	13 (17.3)			0.5 (0.1–1.9)	0.2 (0.02–1.5)
Gly_{16}/Glu_{27} + Arg_{16}/Gln_{27}	18 (26.5)	25 (33.3)			0.9 (0.3–2.6)	0.2 (0.05–1.3)
Gly_{16}/Gln_{27} + Arg_{16}/Gln_{27}	12 (17.6)	15 (20.0)			1.1 (0.3–3.2)	0.1 (0.01–0.8)
Arg_{16}/Gln_{27} + Arg_{16}/Gln_{27}	10 (14.7)	6 (8.00)			2.2 (0.6–8.1)	0.5 (0.07–3.9)
Gly_{16}/Gln_{27} + Gly_{16}/Gln_{27}	12 (17.6)	1 (1.30)			**16.3 (1.8–145)**[*]	9.0 (0.5–161)

Values are shown as number (%). Chi-square (χ^2) for trend was used. OR (95% CI): odds ratio and confidence interval. [&]Adjusted for confounding factors. [*]Statistically significant at $P < 0.05$.

Table 4: Haplotype frequencies of *ADRB2* gene polymorphisms in patients and controls according to the copy number.

Variables	Patients ($n = 68$)	Controls ($n = 75$)	χ^2	P value	Crude OR (95% CI)	Adjusted[&] OR (95% CI)
Arg_{16}/Gln_{27}						
Negative	28 (41.2)	29 (38.7)	0.146	0.703	1.0	1.0
1 copy	30 (44.1)	40 (53.3)			0.7 (0.3–1.5)	0.4 (0.1–1.66)
2 copies	10 (14.7)	6 (8.0)			1.7 (0.5–5.3)	0.5 (0.07–3.9)
Gly_{16}/Gln_{27}						
Negative	39 (57.4)	46 (61.4)	3.378	0.066	1.0	1.0
1 copy	17 (25.0)	28 (37.3)			0.7 (0.3–1.4)	0.3 (0.1–1.08)
2 copies	12 (17.6)	1 (1.3)			**14.1 (1.7–113)**[*]	**20.4 (1.4–296)**[*]
Gly_{16}/Glu_{27}						
Negative	34 (50.0)	22 (29.3)	4.012	**0.045**[*]	1.0	1.0
1 copy	23 (33.8)	38 (50.7)			**0.3 (0.1–0.8)**[*]	0.5 (0.17–1.5)
2 copies	11 (16.2)	15 (20.0)			0.4 (0.1–1.2)	1.9 (0.47–8.2)

Values are shown as number (%). Chi-square (χ^2) for trend was used. OR (95% CI): odds ratio and confidence interval. [&]Adjusted for confounding factors. [*]Statistically significant at $P < 0.05$.

Table 5: Disease characteristics of patients according to *ADRB2* genotypes ($n = 68$).

Characteristics	Arg16Gly genotypes			*P* value	Gln27Glu genotypes			*P* value
	Arg_{16}/Arg_{16}	Arg_{16}/Gly_{16}	Gly_{16}/Gly_{16}		Gln_{27}/Gln_{27}	Gln_{27}/Glu_{27}	Glu_{27}/Glu_{27}	
Total number	10 (14.7)	30 (44.1)	28 (41.2)		34 (50.0)	23 (33.8)	11 (16.2)	
Risk factors for CVD								
Age at diagnosis	52 ± 7.4	53 ± 8.1	55 ± 6.6	*0.577*	53 ± 7.7	55 ± 7.7	53 ± 5.3	*0.367*
Male gender, %	7 (15.5)	22 (48.9)	16 (35.6)	*0.412*	22 (44.0)	21 (42.0)	7 (14.0)	*0.059*
Smokers, %	4 (10.8)	20 (54.1)	13 (35.1)	*0.185*	18 (48.7)	15 (40.5)	4 (10.8)	*0.278*
FH of CVD, %	7 (20.0)	16 (45.7)	12 (34.3)	*0.325*	21 (60.0)	10 (28.6)	4 (11.4)	*0.219*
Hypertension, %	10 (20.8)	20 (41.7)	18 (37.5)	*0.085*	**32 (66.7)**	**12 (25.0)**	**4 (8.30)**	***<0.001****
DM, %	**2 (6.25)**	**10 (31.3)**	**20 (62.5)**	***0.003****	14 (43.7)	11 (34.4)	7 (21.9)	0.429
BMI, Kg/m^2	32 ± 3.8	30 ± 4.0	29 ± 3.6	*0.080*	30 ± 3.3	29 ± 4.1	28 ± 2.9	*0.195*
Lipid profile								
Hyperlipidemia, %	10 (16.1)	28 (45.2)	24 (38.7)	*0.336*	31 (50.0)	20 (32.3)	11 (17.7)	*0.455*
Total cholesterol, mg/dL	202 ± 33	208 ± 38	188 ± 43	*0.085*	199 ± 41	207 ± 40	181 ± 32	*0.139*
Total triglyceride, mg/dL	228 ± 45	200 ± 74	195 ± 54	*0.121*	199 ± 56	199 ± 81	218 ± 38	*0.199*
HDL-C, mg/dL	38 ± 5.2	39 ± 6.7	37 ± 8.7	*0.374*	38 ± 7.7	39 ± 8.0	36 ± 4.8	*0.883*
LDL-C, mg/dL	119 ± 30	1129 ± 37	111 ± 46	*0.125*	122 ± 43	128 ± 39	101 ± 35	*0.099*
VLDL-C, mg/dL	46 ± 8.9	40 ± 15	39 ± 11	*0.121*	40 ± 11	40 ± 16	44 ± 7.5	*0.199*
Cardiac enzymes								
CK, ng/mL	1624 ± 1422	1499 ± 1654	1912 ± 2149	*0.746*	1821 ± 2088	1717 ± 1735	1215 ± 1078	*0.791*
CK-MB. ng/mL	362 ± 412	328 ± 336	294 ± 402	*0.306*	294 ± 320	450 ± 470	126 ± 107	*0.089*
LDH, IU/L	1666 ± 603	1368 ± 1161	1733 ± 1437	*0.289*	1775 ± 1517	1301 ± 882	1451 ± 625	*0.506*

Values are shown as mean ± standard deviation or number (percentage). CVD: cardiovascular disease; DM: diabetes mellitus; BMI: body mass index; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; CK: total creatine kinase; CK-MB: creatine kinase isoenzyme MB fraction; LDH: lactate dehydrogenase. Two-sided chi-square and Kruskal-Wallis tests were used.
*Statistically significant at $P < 0.05$.

for Gly_{16}/Glu_{27}. The absence of Arg_{16}/Glu_{27} was consistent across several haplotype studies conducted in different ethnic populations, including African Americans, Brazilians, Europeans, Asians, and Hispanic-Latinos populations [35, 36, 63–69]. Strong linkage disequilibrium (LD) exists between these two SNPs, and, as a result, the Arg_{16} allele is typically seen with Gln_{27}, whereas Gly_{16} can coexist with either Gln_{27} or Glu_{27} [33, 59, 60, 63, 70].

In the present study, the distribution of Arg16Gly genotypes and alleles did not differ significantly between cases with MI and control subjects. This was in agreement with others who reported no effect of Arg16Gly polymorphism with the occurrence of MI disease among Americans, Europeans, and Turkish populations [15, 19, 25, 71]. However, for Gln27Glu polymorphism, our results showed higher frequency of the Gln_{27} variants among patients and that the Glu_{27} allele conferred protection against the occurrence of MI disease. This was similar to previous studies which reported a significant association of Gln_{27} homozygotes with coronary events, ventricular tachyarrhythmias, and sudden cardiac death [14, 15, 27, 32, 72]. Others highlighted the protective role of Glu_{27} allele against MI in young and elder populations [14, 19, 44, 71]. Also, Kaye et al. showed that subjects with the Glu_{27} allele had a significant improvement in left ventricular ejection fraction compared with subjects homozygous for Gln_{27} [73]. In contrast, one study found the opposite correlation; carriers of Glu_{27} allele had a higher incidence of coronary artery disease and a higher likelihood of later need for coronary revascularization [74], while others did not reveal such associations with MI [11, 13, 23, 75].

The Arg16Gly and Gln27Glu polymorphisms are known to alter the functional properties of the receptor and its behavior after agonist exposure [38, 76]. Although these two amino terminal polymorphisms are located near the ligand-binding site, they do not alter the binding capacity of endogenous or exogenous catecholamines to β_2-AR or affect further G-protein coupling and adenylyl cyclase activation [35, 77]. In addition, receptor synthesis rates and agonist-promoted internalization were not different between the receptors [78, 79]. However, studies of agonist stimulation in cultured cells demonstrated that the Glu_{27} receptor exhibited enhanced resistance to downregulation when compared with the Gln_{27} variant, as assessed by receptor number [77, 80]. Other *in vitro* studies proposed that this desensitization phenomenon might be due to differential alterations in receptor degradation after the internalization step [78, 81, 82]. Moreover, another difference was demonstrated by cell-signaling studies in two cell lines; the Glu_{27} variant was associated with magnification of the catecholamine-induced activation of intracellular signaling transduction [83], thus likely promoting superior cardiovascular performance in human [84].

To examine possible additive or synergistic effects of the two tested polymorphisms, haplotype analysis within the *ADRB2* locus was done, revealing significant difference

Table 6: Disease characteristics of patients according to *ADRB2* haplotypes ($n = 68$).

Characteristics	Arg_{16}/Gln_{27}		P value	Gly_{16}/Gln_{27}		P value	Gly_{16}/Glu_{27}		P value
	Positive	Negative		Positive	Negative		Positive	Negative	
Total number	40 (59)	28 (41)		29 (42.7)	39 (57.3)		34 (50)	34 (50)	
Risk factors for CVD									
Age at diagnosis	53 ± 7.9	55 ± 6.6	*0.355*	53.8 ± 7.8	53.3 ± 7.1	*0.582*	54.3 ± 7.1	52.7 ± 7.7	*0.443*
Male gender, %	29 (64.4)	16 (35.6)	*0.188*	16 (35.6)	29 (64.4)	*0.098*	25 (55.6)	20 (44.4)	*0.200*
Smokers, %	24 (64.9)	13 (35.1)	*0.269*	12 (38.7)	19 (61.3)	*0.548*	15 (48.4)	16 (51.6)	*0.808*
FH of CVD, %	17 (51.5)	16 (48.5)	*0.234*	13 (39.4)	20 (60.6)	*0.598*	20 (60.6)	13 (39.4)	*0.089*
Hypertension, %	30 (62.5)	18 (37.5)	*0.340*	24 (50.0)	24 (50.0)	*0.058*	**16 (33.3)**	**32 (66.7)**	***<0.001****
DM, %	**12 (37.5)**	**20 (62.5)**	***0.001****	17 (53.1)	15 (46.9)	*0.100*	18 (56.3)	14 (43.7)	*0.331*
BMI, Kg/m^2	30.2 ± 4	28.5 ± 3	*0.121*	*29.1* ± 3	29.7 ± 4	*0.955*	28.9 ± 4	30.0 ± 3	*0.090*
Lipid profile									
Hyperlipidemia, %	38 (61.3)	24 (38.7)	*0.184*	25 (40.3)	37 (59.7)	*0.213*	31 (50.0)	31 (50.0)	*1.000*
Total cholesterol, mg/dL	**206 ± 36**	**188 ± 42**	***0.030****	192 ± 44	204 ± 36	*0.070*	198 ± 39	199 ± 41	*0.731*
Total triglyceride, mg/dL	207 ± 68	196 ± 54	*0.945*	192 ± 59	210 ± 65	*0.330*	205 ± 71	199 ± 56	*0.864*
HDL-C, mg/dL	38.5 ± 6.3	37.1 ± 8	*0.170*	38 ± 8.7	38.2 ± 6	*0.471*	38.0 ± 7.1	37.9 ± 7	*0.917*
LDL-C, mg/dL	126 ± 36	112 ± 46	*0.061*	115 ± 48	124 ± 34	*0.133*	119 ± 39	121 ± 43	*0.722*
VLDL-C, mg/dL	41.4 ± 13	39.2 ± 11	*0.945*	38.4 ± 11	42.1 ± 13	*0.330*	41.1 ± 14	39.9 ± 11	*0.864*
Cardiac enzymes									
CK, ng/mL	1530 ± 1583	1912 ± 2149	*0.842*	1978 ± 2359	1471 ± 1305	*0.995*	1554 ± 1554	1821 ± 2088	*0.581*
CK-MB. ng/mL	337 ± 351	294 ± 402	*0.159*	327 ± 405	314 ± 347	*0.660*	345 ± 418	293 ± 320	*0.835*
LDH, IU/L	1442 ± 1051	1733 ± 1437	*0.852*	1655 ± 1662	1493 ± 768	*0.267*	1350 ± 801	1774 ± 1517	*0.358*

Values are shown as mean ± standard deviation or number (percentage). CVD: cardiovascular disease; FH: family history; DM: diabetes mellitus; BMI: body mass index; FBS: fasting blood sugar; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; CK: total creatine kinase; CK-MB: creatine kinase isoenzyme MB fraction; LDH: lactate dehydrogenase. Two-sided chi-square and Mann-Whitney U tests were used.
*Statistically significant at $P < 0.05$.

in the distribution frequencies of haplotype combinations between MI patients and healthy controls. Homozygotes for Gly_{16}/Gln_{27} haplotype showed genetic susceptibility for MI in our population samples, whereas heterozygote individuals with other haplotype combinations (Gly_{16}/Gln_{27} + Arg_{16}/Gln_{27} or Gly_{16}/Gln_{27} + Gly_{16}/Glu_{27}) did not show a significant association with disease risk, thus highlighting a clear dose-response relationship between Gly_{16}/Gln_{27} and MI. Moreover, carriers of Gly_{16}/Glu_{27} haplotype had lower prevalence of MI disease and showed protection against the occurrence of the disease. On the other hand, Arg_{16}/Gln_{27} haplotype did not influence the susceptibility for developing MI in our Egyptian population. Similarly, two clinical studies showed differential distribution of haplotype frequencies of *ADRB2* gene between MI cases and controls in the American population, with evidence showing dominant protective effect of the Gly_{16}/Glu_{27} haplotype on MI and other age-related phenotypes, whereas the presence of any detrimental effect was largely associated with the Gln_{27} allele and Gly_{16}/Gln_{27} haplotype [71, 85]. This was in line with other previous studies which reported significant association between Gly_{16}/Gln_{27} haplotype and low exercise performance in heart failure patients [82]. Consistent with this hypothesis, Gly_{16}/Glu_{27} was shown to be the optimal haplotype combination for cardiovascular response during exercise because of increased receptor numbers and enhanced stroke volume and cardiac output [84, 86] and to elicit more β-blocker drug response in postischemic heart [87]. In contrast, two studies reported a decreased frequency of Gly_{16}/Gln_{27} haplotype in male and female Caucasian patients with MI [13, 88]. Other studies, however, did not find association between the *ADRB2* haplotype and the risk of MI [25, 89, 90]. We propose two explanations underlying the association of Gly_{16}/Gln_{27} haplotype homozygosity with MI development. Several lines of evidence indicated that *ADRB2*, like other G-protein-coupled receptors, form dimers. The formation of β_2-AR dimers was shown to have functional effects on receptor stimulated adenylate cyclase activity [91]. So one possible mechanism is the interaction between two receptors with Gly_{16}/Gln_{27} haplotype forming "homodimer" may have different agonist binding, signal transduction, or agonist-promoted desensitization properties than other types of dimers. However, there is an alternative possibility which is that Arg16Gly and Gln27Glu polymorphisms might be in LD with other polymorphic loci in *ADRB2* gene or nearby inflammatory mediator genes, which have direct effect on the pathogenesis of MI [92]. Drysdale et al. described other molecular haplotypic structure of *ADRB2* including our SNPs [66]. One of them, Arg19Cys polymorphism in the 5′-leader cistron, was found to be in LD with Gln27Glu polymorphism [93] and caused altered receptor translation, hence affecting β_2-AR protein expression *in vitro* [32, 94, 95].

Another main finding in our study was the difference in genotype distribution of the *ADRB2* Gln27Glu polymorphism between hypertensive and normotensive MI patients. Homozygotes of the Gln_{27} variant had a significant higher

frequency of hypertension compared to other genotype carriers. This was in agreement with previous studies conducted in Americans, European, and Han Chinese populations [11, 92, 95]. Binder et al. reported association between both Gln_{27} haplotypes (Gly_{16}/Gln_{27} and Arg_{16}/Gln_{27}) and high diastolic blood pressure in Caucasian males [96]. Subjects with the Gln_{27} variant were shown to have attenuated vasodilatory response to infused isoproterenol, while those with Glu_{27} variant had more robust reversal of constriction than did the Gln_{27} in normotensive males [87, 97]. Furthermore, carriers of the allele Glu_{27} and Gly_{16}/Glu_{27} haplotype showed increased forearm vasodilatation during mental stress and handgrip exercise [98] and might cause arterial hypotension with thoracic epidural anesthesia [99], presumably due to its minimal downregulation by catecholamines, hence increasing β_2-AR signaling [87]. In contrast, others demonstrated that the variation within codons 16 and 27 of *ADRB2* gene were unlikely to confer genetic susceptibility for hypertension in Americans, black South Africans, and Asians suggesting LD with other unidentified genetic variants [48, 56, 64, 100–102].

Assessment of other cardiovascular risk factors in our MI patients revealed a significant difference in genotype frequencies of *ADRB2* Arg16Gly polymorphism between diabetic and nondiabetic MI patients. The frequency of diabetes mellitus (DM) was significantly higher in patients with Gly_{16}/Gly_{16} genotype than other genotypes. The β_2-AR are known to be expressed in a variety of tissues including pancreatic beta-cells and to play a key role in the regulation of glucose metabolism as well as insulin sensitivity and secretion [103, 104]. Masuo et al. have reported significant association between the Gly_{16} variant of the *ADRB2* gene with increased insulin resistance [105]. Prior et al. suggested that *ADRB2* haplotypes mediate insulin action, glucose tolerance, and potentially risk for type 2 diabetes mellitus in obese, postmenopausal women [104]. On the contrary, homozygosity of Arg_{16} allele in the *ADRB2* gene was associated with a higher frequency for development of type 2 diabetes in Taiwanese and Danish populations [106, 107], while no association with type 2 diabetes was found in Koreans [108], Tongan population [109], and young Danish males [110].

In our MI patients, Arg_{16}/Gln_{27} haplotype had higher total cholesterol levels compared to their counterpart. Earlier studies have suggested that the Arg16Gly polymorphism may be associated with cholesterol metabolism in certain populations [39]. In Han Chinese population, Arg_{16} homozygotes had higher serum total cholesterol, triglycerides, and low-density lipoprotein cholesterol levels [111]. An inverse association was found in Saudian population with higher levels of total cholesterol, triglycerides, and LDL-C in Arg_{16}/Gly_{16} and Gly_{16}/Gly_{16} individuals [16]. In addition, the Gly_{16} homozygotes had a lower HDL-C level than the Arg_{16} homozygotes in Japanese population [27], while no association with dyslipidemia was found with *ADRB2* gene polymorphisms at codons 16 and 27 in Korean population [108].

The inconsistent associations of *ADRB2* variants in our results with some prior studies could be caused by the genetic heterogeneity among different ethnic groups [59, 66, 112], since the susceptibility to MI is affected by multiple genetic factors and genotype-environment interactions (multifactorial inheritance mode), in which each factor has a small influence on the development of the disease [4, 113]. Thus variations in the frequency of SNPs in different populations, the modulating effects of other SNPs or mutations within individuals, variation in the penetrance of a SNP due to other factors as age-effect, different gender of enrolled study populations, and the difficulty in matching for all known environmental factors that predispose to MI could provide other plausible explanations for the conflicting results between studies. Overall, our study had few limitations; our sample size may be considered small, there was no control for chronic pharmacological treatment which may interfere with our findings, and only two polymorphisms were analyzed. In addition, the Egyptian population is admixed and a region with a specific genotype combination associated with risk may also be associated with a peculiar environmental factor.

5. Conclusion

In conclusion, the present study provides the first preliminary evidence that Gln27Glu polymorphism of β_2-adrenergic receptors, but not the Arg16Gly polymorphism, is related to the prevalence of myocardial infarction disease in Egyptian population. Identification of the molecular mechanisms underlying the relationship between β_2-adrenergic receptor genes and myocardial infarction offers opportunities to identify individuals at high risk and may help to improve the prevention and treatment of this important disease.

Conflict of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interests.

Authors' Contribution

Eman Toraih, Mohammad H. Hussein, and Dahlia I. Badran conceived and designed the experiments. Dahlia I. Badran collected samples and data. Eman Toraih, Mohammad H. Hussein, and Dahlia I. Badran performed the experiments. Eman Toraih and Dahlia I. Badran analyzed the data. Eman Toraih, Mohammad H. Hussein, and Dahlia I. Badran contributed reagents/materials/analysis tools. Eman Toraih, Mohammad H. Hussein, and Dahlia I. Badran wrote the paper. Eman Toraih, Mohammad H. Hussein, and Dahlia I. Badran were responsible for critical revision and final approval.

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