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Relationship between Genetic Polymorphisms of ß2 Adrenergic Receptor and diabetes associated coronary artery dieses in East Azerbaijan Province of Iran

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ABSTRACT

Pharmacokinetics involves complex connections of gene products affecting pharmacodynamics and Pharmacokinetics. The major allele homozygotes and minor allele carriers of each polymorphism are respectively associated with efficacy enhancement and loss, making the possibility for genotype arrangement interactions that can be measured by clinical trial methodology. But there is little information on the interaction of multiple genetic modifiers of drug response. Pharmacogenomics can be used as a tool for stratified pharmacological therapy in new decade medicine. We investigated whether a predefined combination of the Arg389Gly polymorphism in the adrenergic b1-receptor gene (ADRB1) and the Gln27Glu polymorphism in the adrenergic b2-receptor gene (ADRB2) could predict survival in beta blocker- and metoprolol-treated chronic diabetic patients that suffer from heart failure.

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INTRODUCTION

Heart failure (HF) is categorized by neuro-hormonal-stimulation of the sympathetic nervous system (SNS) and renin–angiotensin–aldosterone system in answer to a descent in cardiac efficiency [1;2]. Unnecessary activation of these systems results in hemodynamic changes such as systemic vasoconstriction and rises in sodium/water retention, and ventricular remodeling, the latter of which is primarily responsible for the advanced nature of the disease. Factually, β-blockers were contraindicated in patients with HF due to their negative inotropic effect. However, as the neuro-hormonal example of HF was valued, there was increasing interest in the β-blockers [3-6]. B-Blockers are competitive antagonists at the β-adrenergic receptors (β-AR), thereby dropping the level of SNS activation. From mid-1990s to early 2000s, several innovative studies established that the β-blockers metoprolol, bisoprolol, and carvedilol reduce morbidity and mortality compared to placebo, when added to standard background therapy that included angiotensin–converting enzyme (ACE) inhibitor therapy .Based on these trial data, these β-blockers are recommended by the consensus HF managingstrategies as vital therapy for patients who have systolic HF and lack contraindications to β-blocker therapy[7;8]. Genetic determination of drug action, or pharmacokinetics, involves complex interactions of gene products affecting pharmacodynamics and pharmacokinetics. These interactions are difficult to examine and quantify, in part because of the ambiguity of the modifier mechanism and the inaccuracy of measuring drug clinical responses.The pharmacological effects of β-blockers originate from their capability to antagonize the β-ARs. Therefore, the genes for these receptors have been a principal focus in β-blocker pharmacokinetic studies. In the cardiovascular system, there are two β-ARs that β-blockers can antagonize: β1-AR and β2-AR, both of which are members of the G-protein coupled receptors superfamily. The β1-AR gene (ADRB1), consisting of 2,860 bp, is located in chromosome 10q24–26. It encodes a 51.3 kDa protein, with 477 amino acid residues [9-11]. B1-ARs are primarily found in the heart, controlling contractility, and heart rate. B2-ARs are more broadly distributed including expression in the heart, respiratory smooth muscle, kidney, and brain, among others. The gene consists of one exon with 2,033 bp located at chromosome 5q31–q32[12]. There are 13 validated SNPs in the ADRB1, which have been reported to the National Center for Biotechnology Information Single Nucleotide Polymorphism database (dbSNP). Of these SNPs, two polymorphisms have been extensively studied both in vitro and in vivo: Ser49Gly (nt 34552562 A>G on NT_030059, rs1801252) and Arg389Gly (nt 34553582 C>G

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on NT_030059, rs1801253).The aim of the present study was to investigate whether ADRB2 polymorphisms at amino acids 16 and 27 might modify relative risk for the development of HF in East Azerbaijan Province of Iran diabetic coronary artery diseases patients.

MATERIAL AND METHODS

Venous blood samples and DNA purification:

From each case, 1 ml venous blood was collected. The 160cases were selected. (Myocardial infarction and negative diabetes history, n=76 and myocardial infarction and positive diabetes history, n=81). Informed consent was obtained from all volunteers. Samples were divided into 500µl aliquots in Eppendorf tubes, and kept at $-20^{\circ C}$ until required. Leukocyte genomic DNA was extracted directly from the blood samples using a QIAamp Blood Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol.

Detection of ADRB2 Polymorphisms at codon 16:

GenomicDNA (120 ng) was amplified in 2X PCRMaster Mix (0.2 U/µl Taq DNA polymerase; 0.4mMdATP, dCTP, dGTP, and dTTP; 2.0 mM MgCl2; Tris-HCl pH 8.5, (NH4)2S04, 4mM MgCl2, 0.2% Tween®20; Ampliqon, Denmark) containing nuclease-free water in a total volume of 20µl, with PCR primers at a concentration of 0.3µM. A previously reported PCR primer set for ADRB2 [10;13]. Amplification of this region was performed with a Multigene OPTImaxLabnet PCR system (Labnet, USA) using an initial denaturation step of 94 ◦C for 5 min; 15 cyclesof 94◦C for 30 s, 60◦C for 30 s, 20 cyclesof 94◦C for 30 s, 57◦C for 30 s, 72◦C for 50s.The amplified PCR products (913bp for the ADRB2, 16 region) was analyzed on a 3% agarose gel with a 50 bp ladder (Fermentase, Carlsbad, CA) as a molecular weight marker (Fig 1).

Statistical Analysis:

The results for continuous variables are expressed as means (SD). Mean results for the groups were compared by the t test, and the statistical significance of differences in frequencies of variants between groups was tested using the $\chi 2$ test. In addition, odds ratios (ORs) and 95% CIs were calculated as a measure of the association between ADRB2alleles and diabetic CAD. Genotype frequencies in patients and controls were tested for Hardy-Weinberg equilibrium and any deviations between observed and expected frequencies were tested for significance using the $\chi 2$ test. Differences were considered significant for P values of less than .05. Data were analyzed using SPSS software, version 17.

Results:

Characteristics of diabeticCAD Patients and Controls:

Of the 76case in the myocardial infarction and negative diabetes history group, there were 53% boys and 47% girls, with a mean age of 59.0 (2.7) years. In the othergroup (myocardial infarction and Positive diabetes history group, there were 48% boys and 52% girls, with a mean age of 56 (3.1) years (Table 1).

Table 1: Demographic characteristics of the subjects.

	Characteristics of cases and Controls	
	CAD and Positive Diabetes history (n=81)	CAD and Negative Diabetes history (n=76)
Age, mean (SD), y	56(3.1)	59(2.7)
Men, % of patients	48	53
women, % of patients	52	47
Smoking status (%)	46	56
Body mass index (kg/m2)	23.6+/- 0.1	20.3+/- 0.1
Hyperlipidemia (%)	26.7	18.8
Family history of premature CAD (%)	12.7	11.1

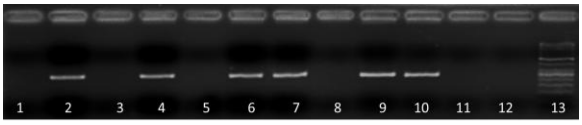

Fig. 1: Lines 1, 2, 3, 4 and 5, 6 show913bp for the ADRB2, 16regions in Arg/Arg genotype.Lines 7, 8 show913bp for the ADRB2, 16regions in Gly/Gly genotype. Lines 9, 10 shows 913bp for the ADRB2, 16 region in Arg/Gly genotype. Lines 11, 12, shows non template control and 13^{th} line shows 250bp size marker, respectively.

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Genotype Distributions and Allele Frequencies of ADRB2 Arg/Gly Polymorphisms at Codon 16 in Patients and Controls

ADRB2 16 genotype frequencies conformed to the Hardy- Weinberg equilibrium in both patients (p=.23) and controls (p=.12). In the case group, the frequencies were 29.6% for Arg/Arg homozygous individuals, 48.1% for Arg/Gly heterozygous individuals, and 20.90% for Gly/Glyhomozygous individuals, while in the control group they were 46.05%, 27.6%, and 26.3% respectively (Table 2).

Table 2: Genotype Distributions and Allele Frequencies of ADRB2 Arg/Gly Polymorphisms at Codon 16 in CAD Patients with diabetes and Controls.

Polymorphic Site	CAD and Positive Diabetes history (n=81) No.(%)	CAD and Negative Diabetes history (n=76) No.(%)	OR (95% CI)	p Value
ADRB2 16				
Arg/Arg	24(29.6)	35(46.05)		
Arg/Gly	39(48.1)	21(27.6)	1.35 (0.7-2.4)	0.230
Gly/Gly	17(20.9)	20(26.3)	1.65 (0.9-3.2)	0.12

The estimated frequencies of various haplotypes in both the control and patient groups and the significance of the association with CAD are analyzed. The association was no significant in all cases.

Discussion:

Data from numerous studies suggest that there is a genotype group that responds less favorably to β-blocker therapy. However, given the consensus-guideline driven use of β-blockers, it is hard to predict suppressionβ-blocker therapy based on genetic information. Thus, it is unlikely that in the future we will withhold β-blocker therapy based on genotype [14-18]. Two common ADRB2 polymorphisms—Arg16Gly and Gln27Glu—have been studied for their possible association with CAD-related phenotypes. However, contradictory data exist regarding their clinical significance and effect in different populations and different cases [19-22].Several studies have been performed focusing on different AR genotypes and ethnicity, and ample differences in allele frequencies between the Caucasian and the AfricanAmerican population have been demonstrated. A general theory is that the African American people are less responsive to βAR blocker therapy than Caucasian patients because genotypes with poor βAR blocker response are more common in African American patients [23-28]. The drug management resulted in asignificantly more pronounced reduction in exercise heart rate in Caucasians than in African Americans. Self-sufficiently, the Arg389 allele was associated with a greater heart rate reduction as well. However, the ethnic differences in heart rate reduction were still apparent even afterthe investigators adjusted for the different genotype spreading.This brought them to the conclusion that there are further, yet unidentified, factors contributing to the ethnicdifferences in heart rate response to βAR blockers[29]. Certain haplotypes of the ADRB2 gene may play an important role in modifying clinical characteristics of CAD phenotypes. Although the precise associations have not been well studied, some researchers have said that assessment of individual SNP effects without consideration of haplotypes may have resulted in unpredictable associations because the SNPs in the ADRB2 gene are tightly related. On examining the association between Arg16Gly and haplotypes in relation to diabeticCAD, we found no significant association. Future studies are recommended to understand the possible role of these genetic variations in regulating responses toCAD therapy in the East Azerbaijan Province of Iran population.

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