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Genetic polymorphism in *ADRB-1* is associated with type 2 diabetes susceptibility in Iranian population

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ABSTRACT

β_1-Adrenegic receptor (ADRB-1) leads to increase both renin and ghrelin secretions which are considered to be two hormones associated with type 2 diabetes mellitus (T2D) and insulin resistance. So, to clarify the possible impact of ADRB-1 variant in T2D, we investigated rs1801253C/G (Arg38Gly) polymorphism in a sample of Iranian population. This case-control study was carried out in 500 unrelated Iranian subjects that used Tetra amplification refractory mutation system polymerase chain reaction (Tetra ARMS-PCR) for genotyping rs1801253C/G variant. The finding demonstrated a strong association between rs1801253C/G polymorphism and T2D in versus inherited models (GG vs. CC, OR = 2.88, CI = 1.44–5.78, $p = 0.003$; G vs. C OR = 1.87, CI = 1.31–2.67, $p = 0.0006$; CG + GG vs. CC OR = 1.64 CI = 1.06–2.500 $p = 0.024$; and GG vs. CC + CG OR = 2.78, CI = 1.41–5.56, $p = 0.0021$). Our findings suggest that *ADRB-1* rs1801253C/G polymorphism was prominently associated with T2D in Iranian subjects. These remarkable results needed to evaluate in other investigations with larger sample sizes and different ethnicities to confirm.

1. Introduction

Type 2 diabetes (T2D) is one of the most important metabolic disorder affecting thousands of people every year worldwide (Legro et al., 1999). However, Although the mechanisms underlying T2D pathogenesis are found to be closely related to insulin secretion and response of target cells to insulin, it has been well established that genetic factors play a pivotal role in the progression and development of T2D (Hara et al., 2002; Dupuis et al., 2010).

The β_1-adrenegic receptor (ADRB-1) is one of the members of the G-coupled receptor which its natural agonists are epinephrine and norepinephrine. These catecholamines start interaction signaling via adenylyl cyclase activation (Johnson et al., 2003). Previous reports demonstrated this receptor is expressed in different tissue such as adipocytes leading to lipolysis promotion and cardiac myocytes, resulting in contractility and increase in heart rate (Engelhardt et al., 1999; Jahns et al., 1999; Nikolaev et al., 2006). Regarding *ADRB-1* and T2D, this hypothesis is planned that this gene may be associated with insulin sensitivity (Mottagui-Tabar et al., 2008; Burguete-García et al., 2014), energy balance (Dionne et al., 2002; Mottagui-Tabar et al., 2008), and also linked to T2D's complications such as obesity (Li et al., 2006), cardiovascular diseases (Johnson and Liggett, 2011) and hypertension (Shioji et al., 2004; Gjesing et al., 2007) but we consider this gene as a candidate for T2D susceptibility because of the influence of *ADRB-1* on the renin and ghrelin secretions that these two hormones were in a close relation with T2D (Pöykkö et al., 2003a; Pöykkö et al., 2003b; Scheen, 2004a; Petersen et al., 2012; Mani et al., 2016). As shown in Table 1, rs1801253C/G as a common variant of the *VDRB-1* gene is a single nucleotide polymorphism (SNP) with a missense mutation that causes amino acid exchange (Arg → Gly). This SNP

Abbreviations: T2D, Type 2 diabetes mellitus; ADRB-1, β_1-adrenegic receptor; SNPs, Single nucleotide polymorphisms; Tetra ARMS-PCR, Tetra amplification refractory mutation system polymerase chain reaction; CI, Confidence Intervals; OR, Odds Ratio; FBS, Fasting blood sugar; TC, Total cholesterol; TG, Triglyceride; BMI, Body mass index; HDL-C, High density lipoprotein-cholesterol; LDL-C, Low density lipoprotein-cholesterol; cAMP, Cyclic adenosine monophosphate; Arg, Arginine; Gly, Glycine; F, Female; M, Male

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Table 1
Information of the variant of *ADRB-1* gene.

dbSNP	Chromosome	Functional Consequence	Amino acid exchange	Chromosome position	Allele (major/minor)	MAF	Heterozygosity
rs1801253	10	missense variant	Arg389Gly	114,045,297	C/G	0.2983	0.389

SNP: single nucleotide polymorphism, MAF: minor allele frequency.

Table 2
The primers used for detection of single-nucleotide polymorphism in *ADRB-1* rs1801253C/G.

Primers	Sequence (5′ to 3′)	Annealing temp.
F_O	CTTCCACAGTGAGCTGGTGCCAGAC	56 °C
R_O	TGTCCCCTACTACATCGTCATCGTC	
F_I (C allele)	CCTGAGCACGCAGCAGAGCAGACC	
R_I (G allele)	CCTGAGCTCGCAGCAGAGCAGACC	

influenced on the ability of the receptor to bind the G_s molecule (Podlowski et al., 2000).

To the best of our knowledge, the effect of ADRB1 Arg389Gly variant on the T2D risk in a sample of Iranian population has not yet been investigated, thus, the current study was aimed to access possible association between rs1801253C/G (Arg389Gly) variant and risk of T2D.

2. Materials and methods

2.1. Patients

The current case-control study conducted on 250 T2D patients from Diabetic Center, Bu-ali Hospital, Zahedan, Iran as the one and only center for T2D patients in Zahedan. 250 gender- and age-matched healthy control individuals with no history of endocrinology diseases were selected randomly from five hospitals of Zahedan. The American Diabetes Association (ADA) (Association, A.D., 2014) and our previous studies (Saravani et al., 2015; Saravani et al., 2017b) were used for study design and enrollment process.

The local committee of Zahedan University of Medical sciences approved the ethical section of study. All participants signed the informed consent before arrive to study. 5 mL of peripheral blood of both groups was drawn including; 2 mL in CBC tubes (containing EDTA as anticoagulant) for genomic DNA extraction by salting out method and 3 mL in serum-separating tubes for some biomedical indices analysis (Saravani et al., 2017a).

2.2. Genotyping

Genotyping of *ADRB-1* polymorphism was performed by Tetra-ARMS PCR method based on the primer sequences in Table 2. Each PCR reaction tube with 15 μL final volume including: 80–100 ng/mL of genomic DNA (1 μL), 10 pmol/mL of each primer (1 μL) (Pishgam Co., Tehran, Iran), 3 μL of DNase-free water (SinaClon Bio-Science Co., Tehran, Iran), and 7 μL of Master Mix (Ampliqon taq 2× mastermix, Denmark). The PCR conditions were: initial denaturation 95 °C for 7 min, followed by 30 cycles including each cycle: denaturation step at 95 °C for 30 s, annealing step at 56 °C for 25 s following by an extension step at 72 °C for 30 s. After, ending thirtieth cycle, final extension was done for 5 min at 72 °C. The PCR products were electrophoresed based on agarose gel stained by 0.5 μg/mL Ethidium bromide, and visualized under UV light by UV trans-illuminator. Nearly 15% of samples of each group were re-genotyped for quality control of genotyping. The results were completely re-productive.

Table 4
Genotypic and allelic frequencies of *ADRB-1* polymorphism (rs1801253 C/G) in T2D patients and control subjects.

ADRB-1 polymorphism	T2D *n* (%)	Control *n* (%)	OR (95% CI)	*p*-Value
Codominant				
CC	184(73.6)	205(82)		
CG	35(14)	33(13.2)	1.18(0.71–1.98)	0.526
GG	31(12.4)	12(4.8)	2.88(1.44–5.78)	**0.003**
Allele				
C	403(80.6)	443(88.6)		
G	97(19.4)	57(11.4)	1.87 (1.31–2.67)	**0.0006**
Dominant				
CC	184(73.6)	205(82)		
CG + GG	66(26.4)	45(18)	1.64(1.06–2.50)	**0.024**
Recessive				
CC + CG	219(87.6)	238(95.2)		
GG	31(12.4)	12(4.8)	2.78(1.41–5.56)	**0.0021**
Over-dominant				
CC + GG	215(86)	217(86.8)		
CG	35(14)	33(13.2)	1.08(0.64–1.79)	0.79

CI: confidence interval; OR: odds ratio, T2D: Type 2 diabetes, $p < 0.05$ was regarded as statistically significant (bolded *p*-value).

2.3. Statistical analysis

The data were analyzed statistically by SPSS software version 22 (Chicago, IL, USA). The quantity and quality data were achieved by independent sample *t*-test and the x^2 test, respectively. The Fisher's exact test was also admired to analyze the genotypes and frequencies of data obtained in this study. The calculation of the frequency of alleles

Table 3
Clinical-demographic characteristics of T2D patients and controls.

	T2D (n = 250) (n ± SD)	Controls (n = 250) (n ± SD)	*p*-Value
Age (year)	54.68 ± 10.19	49.60 ± 9.99	0.554
Sex (Female/male)	173/77	178/72	0.696
FBS (mg/dL)	200.63 ± 100.58	101.41 ± 30.12	< **0.0001**
TC (mg/dL)	187.00 ± 48.15	179.39 ± 36.27	**0.028**
TG (mg/dL)	165.24 ± 81.09	143.13 ± 81.73	0.356
HDL-C (mg/dL)	52.92 ± 19.38	53.41 ± 13.56	**0.020**
LDL-C (mg/dL)	102.59 ± 38.19	101.50 ± 28.74	**0.038**
BMI (kg/m^2)	27.61 ± 5.49	21.54 ± 2.51	< **0.0001**

FBS: fast blood sugar, TC: total cholesterol, TG: triglyceride, HDL-C; high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, BMI; body mass index, T2D: type 2 diabetes, kg: kilogram, m^2: square meter, mg: milligram, dL: deciliter, $p < 0.05$ was regarded as statistically significant (bolded *p*-value).