Frequency of *KCNQ1* Variant rs2237892 in Type 2 Diabetes in East Azerbaijan Population, Northwest of Iran

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Abstract- Genome-wide association studies in Asian population for the first time identified common variants in the *KCNQ1* gene to be associated with type 2 diabetes in Japanese populations, recently. This result has been replied in some other East Asian population but, still now, there is no any report about this subject in Iranian population. The aim of this study was to investigate the frequency of *KCNQ1* variants in type 2 diabetes in East Azerbaijan population, northwest of Iran. A total of 75 T2D and 90 normal East Azerbaijan subjects were entered the study. A demographic date was recorded for all subjects. Genomic DNA was extracted from the whole blood, and the *KCNQ1* single nucleotide polymorphisms (SNPs) rs2237892 were genotyped with PCR-RFLP technique. The results of the study indicated an increased level of FBS and HbA1C among T2D cases (*P*<0/0001), as expected. Screening for *KCNQ1* rs237892 among T2D case group revealed that the genotype frequencies of CC, CT and TT were 87.0% (67/77), 10.4% (8/77) and 2.6% (2/77), respectively. The allelic frequencies of C and T in case subjects were 92.21% (142/154) and 7.79% (12/154), respectively. Analysis of the normal control subjects showed the genotype frequencies of CC, CT and TT were 94.4% (85/90), 3.3% (3/90) 2/2% (2/90), respectively. The allelic frequencies of C and T among controls were 96.11% (173/180) and 3.89% (7/180), respectively. In conclusion, the results of our study indicated that there are no any significant differences in frequency of *KCNQ1* rs237892 alleles between T2D and control subjects.

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Keywords: Type 2 diabetes; GWAS; *KCNQ1* rs237892; Polymorphism

Introduction

Diabetes mellitus is the most common metabolic disorder, and there are now 415 million adults aged 20-79 with diabetes in the world. The prevalence of T2D is progressing, and it is estimated that there will be 642 million until 2040. The prevalence of diabetes in Iranian population is 8.5%, and there are over 4.6 million cases of diabetes in Iran (1).

Recent genome-wide association (GWA) studies showed a significant association of *KCNQ1* polymorphism with type 2 diabetes T2D in Asian population (2,3).

KCNQ1 comprises 19 exons and extends more than 400 kb on chromosome 11p15.5 (4). The gene encodes the pore-forming subunit of the voltage-gated K channel (KvLQT1), which plays a main role in directing the repolarization of the ventricular (5). Mutations in *KCNQ1* have been associated with congenital cardiac disorders, for instance, long QT syndrome and familial atrial fibrillation (6). Further to the heart and cochlea, *KCNQ1* is ubiquitously expressed in epithelial cells including the exocrine and endocrine cells of the pancreas (7). The *KCNQ1* risk alleles for T2D like other diabetes candidate genes seem to be allied with damaged pancreaticβ-cell as evaluated by fasting state and oral glucose tolerance test (OGTT) (3). This research focused on the evaluation of the association of single nucleotide polymorphisms (SNPs) and haplotypes of the *KCNQ1* gene with type 2 diabetes mellitus in East Azerbaijan population in Iran.

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Materials and Methods

Subjects and data collection

In this case-control study, a total of 100 diabetic patients and 100 healthy people were entered the study. Based on the results of biochemical tests in the early stages of the study, 13 case and 10 control subjects were excluded due to lack of inclusion and exclusion criteria.

Inclusion criteria for this study were the age range of 30 to 70 years old and type II diabetes mellitus diagnosed by an endocrinologist with the standards of ADA (American Diabetes Association). The Exclusion criteria include 1- Other types of diabetes; 2- Insulin prescription; 3- Other diseases such as rheumatoid arthritis and 4- Age less than 30 years and above 70 years. In addition inclusion criteria for controls (healthy control subjects) include: no types of diabetes (fasting blood sugar (FBS) is less than 110) and the age range was 30 to 70 years having Exclusion criteria for healthy control group include; 1- History of type 2 diabetes in first grade appointees; 2- GDM; 3-Younger than 30 years and above 70 years. To investigate the polymorphism in patients with type II diabetes and healthy control subjects, 5 cc blood samples were collected after obtaining the written consent form.

Biochemical analyses

Biochemical tests such as FBS, 2hpp Glucose, Tg, Chol, HDL, LDL, A1C, Cr were determined using Hitachi autoanalyzer (Tokyo, Japan).

Genetic analyses

The PCR-RFLP method was used for detection of *KCNQ1* rs2237892 polymorphism. *KCNQ1* SNP rs2237892 sequence was downloaded from the NCBI, and specific primers were designed for this SNP using Gene Runner software. The primers used in this study were produced by Bioneer Co. The Sequences of primers were as follows: F - 5´-CTGTGTGCGTGTGGGGATG- 3´ and R- 5´-CTGTGTGCGTGTGGGGATG-3´. Genomic DNA was extracted from peripheral blood leukocytes with DNG-Plus (Sinaclon, Iran). Nanodrop was used to determine the optical density (OD 260/280) and concentration of purified DNA. The PCR was carried out in a 20 µL reaction containing 10 µL of 2x HotStart MasterMix, 10 pM of forwar and reverse primers, 100 ng of DNA template and 1 unite of Taq DNA polymerase. Amplification was performed in 35 cycles with initial denaturation at 95°C for 5 min, cyclic denaturation at 95°C for 1min, annealing at 62°C for 30 s, extension at 72°C for 20 s, and a final extension at 72°C for 5 min. The PCR product was applied on 1.5 % agarose gel and visualized by safe stain staining.

For RFLP analysis, 200 ng of PCR products were entered in a digestion reaction containing 2 units of Hpa II restriction enzyme in NE Buffer 2.0. The enzyme-digested products were analyzed using 2% agarose gel for 30 min at the voltage 120 V. DNA Maker was molecular weight standard. Gel imaging system was used to judge the genotype results.

Statistical analysis

Gene counting technique was used for calculating genotype and allelic frequencies. The agreement of the tested group with Hardy-Weinberg (H-W) equilibrium was inspected by *Chi*-square test. The quantity data are expressed as mean±SEM. *P* were calculated using an unpaired *t*-test. GraphPad/Prism® 7 for Windows version 7.02 software was used for the statistical treatment of data. In the case of $P<0.05$, the variance had statistical significance.

Results

Biochemical analyses

Seventy-seven T2D and 90 normal East Azerbaijan subjects signed the consent forms and donated blood. The demographic and biochemical parameters of the subjects are depicted in table 1. As shown in table 1, the systolic blood pressure, the serum level of triglyceride and creatinine were higher in T2D subjects whereas diastolic blood pressure, cholesterol and Low-Density Lipoprotein (LDL) in T2D were lower than normal subjects, but the differences were not significant ($P>0.05$).

As expected, the established T2D risk factors (e.g., Body Mass Index (BMI)) were higher in the T2D group than in the controls. A significant difference was also observed between the two groups with respect to the main factors, including Fasting Blood Sugar and HbA1C. In contrast, the concentrations of High-Density Lipoprotein (HDL) and Blood Urea Nitrogen (BUN) were lower in the T2D population than in the controls ($P<0.05$).

Prevalence of *KCNQ1* rs2237892 polymorphism

PCR amplification and RFLP analysis of *KCNQ1* rs2237892 locus showed two bands of 220 bp and 67 bp in CC homozygote genotype; one band of 287 bp in TT homozygote; and three bands of 287 bp, 220 bp and 67 bp in CT heterozygote genotypes (Figure 1).

The risk allele frequencies of rs2237892 in normal subjects were 0.96 versus 0.92 in diabetic patients, respectively (Table 2). Statistical analysis for the risk allele of rs2237892 (C) between T2D and normal subjects showed that the difference was not statistically significance (*P*>0.05, Table 2).

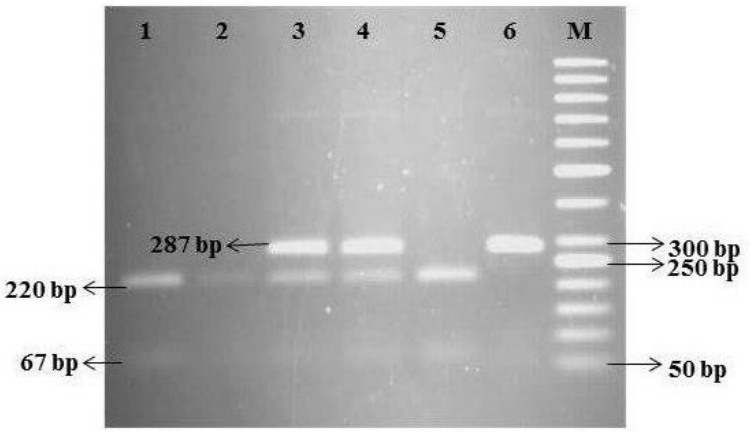

Figure 1. Electrophoretic pattern of PCR-RFLP on *KCNQ1* locus rs2237892
Lane 1, 2 and 5 genotype CC; lane 3 and 4 genotype CT, lane 6 genotype TT and lane M for Marker

Table 1. Demographic and biochemical parameters of study subjects

Parameters		Type 2 diabetes	Normal	*P*
Gender	**Male**	35	44	
	Female	42	46	
High (*cm*)		160/4 ± 1/354, n=77	163/9 ± 1/164, n=90	0/5012
Weight (*Kg*)		77/96 ± 1/704, n=77	78/27 ± 1/441, n=90	0/4144
BMI ($^{Kg}/_{m^2}$)		30/58 ± 0/618, n=77	29/18 ± 0/5035, n=90	0/2484
FBS (*mmHg*)		176/6 ± 8/631, n=75	94/15 ± 1/016, n=81	<0/0001
A1C (*mmHg*)		8/222 ± 0/3058, n=63	5/991 ± 0/1179, n=11	<0/0001
Systolic blood pressure (*mmHg*)		140/4 ± 2/52, n=77	135/3 ± 2/451, n=90	0/6547
Diastolic blood pressure (*mmHg*)		85/77 ± 2/055, n=77	89/18 ± 1/549, n=90	0/0632
Tg (*mmHg*)		203/3 ± 15/25, n=63	187/7 ± 12/39, n=82	0/5169
Chol (*mmHg*)		185/6 ± 5/87, n=64	202/6 ± 5/518, n=82	0/6113
HDL (*mmHg*)		40/58 ± 1/201, n=45	44/63 ± 2/548, n=59	<0/0001
LDL (*mmHg*)		93/91 ± 4/009, n=32	104 ± 3/253, n=56	0/6821
BUN (*mmHg*)		16/66 ± 0/908, n=22	18/65 ± 3/651, n=41	<0/0001
Cr (*mmHg*)		0/9364 ± 0/02326, n=55	0/9174 ± 0/02545, n=69	0/1227

Table 2. Comparison of the genotype and allele frequencies of *KCNQ1* locus rs2237892 among control and case groups

Group	Risk allele (Frequency)		*P*-value	Genotype n (distruction %)			*P*
	C (n%)	T (n%)		CC	CT	TT	
Control	173 96.11%	7 3.89%	0.1247	85 94.4%	3 3.3%	2 2.2%	0.1815
Case	142 92.21%	12 7.79%		67 87%	8 10.4%	2 2.6%	

Discussion

Type 2 Diabetes Mellitus (T2D) is a complicated polygenic or multi-gene disorder with considerable genetic heterogeneity. It is the result of interactions of multiple genes and genetic and environmental factors demonstrating itself by hyperglycemia, due to the impaired insulin secretion or function. So far, various strategies have been attempted to find the candidate genes associated with T2D in different population.

However, studies in different races and regions have shown dissimilar results.

KCNQ1 gene is one of the members of voltage-gated potassium channel family. It is located at the 11^{th}chromosome 11p l5.5, about 404 kb, and is composed of 17 exons. The exons are in the size range of 47–1122 bp (8). *KCNQ1* encodes for a kind of voltage-gated potassium channel protein, which is frequently referred to as KvLQTl. KvLQTl is a membrane potential potassium channel protein, which is composed of 676 amino acid residues (9) and expressed in pancreatic islet INS-1b cell, playing an important role in regulation of insulin secretion (10).

Two principal GWA studies displayed that common genetic variation in *KCNQ1* is associated with type 2 diabetes (2,3). Rs2237892 has been found to be attributed to a fasting parameter of insulin secretion (β-cell function) in a Japanese population and with an OGTT-derived insulin secretion parameter in a European cohort (3).

Based on the results of a recent GWAS, the rate of *KCNQ1* SNPs occurrence in the Asian populations was higher compared to the European populations. Results from studies in Asian countries like China, Malaysia, Korea, Lebanon and some European countries such as Germany and Netherlands demonstrated the relevance of this SNP with T2D (11-16). This study was designed to analyze the correlation of *KCNQ1* candidate polymorphism with T2DM risk in peoples from North West of Iran.

Comparison of T2D risk factors among patients and control subjects revealed that FBS and HbA1C were significantly higher in T2D patients than controls, whereas HDL and BUN in T2D subjects were lower than normal subjects. These findings are consistent with several studies (17,18,23) demonstrating dysregulation of biochemical parameters in T2D.

Analysis of the frequency of rs2237892 among T2D patients in East Azerbaijan showed that the polymorphism was similar to the Tunis Arabs and the Saudi population and the results reported from Malaysians living in Singapore (19-21). Tan *et al*., reported the association between T2D and this SNP in the Chinese and Asian Indian population in Singapore but not in Malays (21); however, the same group later reported that this SNP was associated with T2D in Malays but not in Chinese (22). These contradictory results might be due to the small sample size of diabetic Malays in the first study compared to the second study (100 vs. 1076) in which the association of Rs2283228 with T2D was demonstrated.

In conclusion, our study does not replicate the association of rs2237892 with T2D in East Azerbaijan subjects. These results are consistent with results of previous study by Bazzi *et al*., and Turki *et al*., confirming the variation in prevalence of SNP genotypes between different ethnic groups.

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