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The Soluble Carrier 30 A8 (SLC30A8) Gene Polymorphism and Risk of Diabetes Mellitus Type 2 in Eastern Azerbijan Population of Iran

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Abstract

Type 2 Diabetes Mellitus (T2D) is the most common metabolic disease demonstrating itself by hyper- glycemia, due to impaired insulin secretion or action. Recently, Whole-Genome Association studies have revealed the role of several new genes responsible for T2D. One of the most studied genes is SLC30A8 (Zn-T8) which is exclusively expressed in pancreatic β-cells and participates in insulin storage and transfer. A number of previous studies support the role of R325W (rs13266634) variant of this gene in T2D risk. The present study was designed to determine the possible association of rs13266634 polymorphism of SLC30A8 gene with T2D in the population of Eastern Azerbaijan province. We genotyped the rs13266634 polymorphism in Azeri samples, using 250 samples prepared from T2D case and control individuals in equal numbers. Genotyping were performed using Polymerase Chain Reaction and Restriction Fragment Length Polymorphism assay (PCR-RFLP). No significant association was detected between rs13266634 variant of SLC30A8 gene and T2D in our study population ($p>0.05$). Moreover, none of the metabolic and anthropometric parameters had significant differences for RW (heterozygote mutant) and RR (homozygote mutant) genotypes in case and control groups ($p>0.05$). Significant differences was observed in fasting blood sugar (FBS), glucose tolerance test (GTT), age, education and economic status between case and control groups ($p<0.05$), while the differences in BMI and gender between the two study groups were non-significant.

Keywords: T2D; SLC30A8; Polymorphism

Introduction

Type 2 diabetes mellitus (T2D) is one of the metabolic diseases demonstrated by hyperglycemia related to impaired insulin secretion or activity in pancreatic β cells. T2D is one of the major global public

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health problems. According to the recent reports, its worldwide incidence in adults is about %6 [1].

Around 7.7% of adults aged between 25-64 years in Iran are affected by T2D while one-half of them are unrecognized and 16.8% have impaired fasting blood sugar [2]. The incidence rate of T2D in Iran is 10.6 per 1000 person for every year. This rate changes in urban populations of Iran for more than 1 % [3].

T2D has occupied the sixth position of death causes in order that diabetic patients have twice risk of death comparing their normal counterparts with the same age. The main reason of death is related to diabetes complications [2].

The most common T2D complications include: cardiovascular diseases, retinopathy, nephropathy and diabetic foot that may lead to limb imputation. Thus to prevent the abnormal consequences of the disease and decrease the mortality rate, effective clinical and practical interventions are required to diagnose the major predisposing factors involved in the pathogenesis [4]. Both of genetic and environmental factors have proposed as etiology for T2D. The association of a number of genes including CAPN10, PPARG, KCNJ11 and TCF7L2 with T2D has been confirmed in many population studies with different ethnic origins including Iran [5,6]. A new series of candidate genes have also been assumed by many GWA (Genome Wide Association) studies for European countries [7-10] and other populations [11-13].

SLC30A8 gene located on 8q24.11 is one of susceptibility genes for T2D which has been studied by many research groups in different countries. The gene codes for a protein called Zinc transporter 8 that is a part of solute carrier major family proteins. The major structure of this protein contains six trans-membrane domains accompanying histidine-rich loop between trans-membrane domains IV and V in frame of 369 amino acid residues like other members of cation diffusion facilitator (CDFs) [14-16]. The gene has a predominant expression in pancreatic β-cells with major role of transferring zinc from cytoplasm into insulin secretary vesicles. The mentioned vesicles are specialized in storage of insulin in bounded form with two Zn2+ ions prior to secretion. In addition to insulin secretion, it seems that zinc is involved in translocation and synthesis of insulin [17].

In a study to assess the risk of offspring of diabetic patients being affected by diabetes, Boesgaard et al found that in condition of SLC30A8 gene over expression, there is an increase in glucose induced insulin secretion in insolinoma cells. They found that mutations in the genes on specific sites result in reduced first-phase insulin release in non-diabetic offspring of type 2 diabetes patients. These researches concluded that the protein encoded by SLC30A8 gene has an important role in transferring of insulin within cells. These finding may result in a possible therapeutic approach with zinc supplementation or pharmacological manipulation of its transport within related cells, hence shedding light on important role of genetic elements on synthesis and secretion of insulin [9].

Although a number of studies had been reported about the possible role of SLC30A8 gene polymorphism C/T (R325W) in the pathogenesis of T2D, however the results were controversial as a number of studies were in agreement with the protective effect of W allele against T2DM risk while some others could not find any association between R allele and T2DM risk. In addition different results had been reported about the role of heterozygote genotype (RW) on T2DM risk in various populations hence, there was no published data about our study population. For these reasons we performed an association study on possible relevance of R325W (rs13266634) polymorphism of SLC30A8 gene with T2D in the population of Eastern Azerbaijan province of Iran.

Materials and Methods

A total of 250 samples prepared from case and control groups in equal numbers (case=control=125), were included in our study. The patient and control groups were aged between 40-70 years. The questionnaires including information about gender, age, medication history, anthropometric measurements (body height and weight), FBS and BS 2hr results, education and income level were filled for each individual [Table 1].

To ensure absence of any diabetes vulnerability, all control participants were selected from individuals who had normal GTT (glucose tolerance test), BS 2hr and FBS test, no previous history of diabetes and glucose intolerance in related family members, no radiation or chemotherapy history and absence of known cancer and genetic disorders. Moreover, every pregnant woman was excluded of our study. The diabetic patients which were included in the present study were determined as having fasting plasma glucose concentration >7.0 M mol/l or a plasma glucose concentration >11.1 M mol/l, 2 hr after oral taking of 75g glucose, in agreement with World Health Organization (WHO) diagnostic criteria for type 2 diabetes under confirmation of endocrinologist. The patients affected by other types of diabetes (type 1 and its specific subtypes) or known genetics disorders, history of chemotherapy or radiation, pancreatic diseases, Cushing's syndrome and acromegaly, were omitted from the study.

Table 1. Clinical and metabolic characteristics of patients

Characteristics	T2D(N:125)	Controls(N:125)	p
Age,years	55.5±8.9	52.5±8.3	0.006
Sex(male/female)	64/61	62/63	0.65[a]
Fasting Blood Sugar,mg/dL	97.6±16.5	84.4±11.2	<0.0001
2h Blood Sugar, mg/dL	137.5±15.6	128.6±10.8	<0.0001
BMI, kg/m2	24.01 ±3.31	24.16±3.77	0.73
Education	-	-	0.004[a]
Income	-	-	0.001[a]

[a] χ^2-test

DNA Extraction and Polymerase Chain Reaction (PCR)

Total genomic DNA was extracted from peripheral blood samples based on salting out DNA extraction protocol and the DNA concentration was determined by spectrophotometer. DNA samples with optical density (OD) between 1.6 and 1.8 were selected for the following steps of the project. The isolated DNA was amplified through polymerase chain reaction (PCR) in a total volume of 25 µl using Biorad thermocycler system (Bio-Rad Laboratories, Hercules, CA, USA). The PCR master mix contained 0.75 µl of MgCl2, 1 µl each of forward (5′GAGCAGTCGCCCATGCGTGT3′) and reverse (5′AAGGCAGTCGGGGTCCTGGT3′) primers, 2.5 µl of 10x buffer (tris-Hcl and Kcl), 18 µL ddH2O, 0.5 µM of d NTP mixture and 2 units of Taq DNA polymerase (Cynagen). For each reaction, 100 ng of genomic DNA was used as template.

After an initial denaturation at 94°C for 5 min, 30 cycles of PCR was carried out at 94°C/1 min, 56°C/30 s, 72°C/45 s). A final extension was performed at 72°C for 5 min.

Restriction Enzyme Digestion

All PCR products (10 µl) were digested with 10 unit of PVUII restriction endonuclease at 37°C, overnight.

In the case of C>T base substitution in the SLC30A8 gene, a restriction site is created (5′CAG↓C**T**G3′) which can be recognized by the enzyme and the 181 bp PCR product, is cut into two pieces of 66 and 115 bp.

Polyacryamide Gel Electrophoresis

The amplified PCR product was separated by electrophoresis in %8 polyacrylamide gel colored with %1 AgNO3 (every 3 µL of PCR product mixed with 1µL loading dye). The electrophoresis was conducted at constant voltage of 120 for 150 minutes at 37°C using 1X TBE buffer. RFLP products subjected to %8 polyacrylamide gel electrophoresis used for fractionation of PCR products (5µL of RFLP products were added to 1 µL loading dye for loading).

Statistical Analysis

The data were analyzed using SPSSv.15 as statistical software and Exact Fisher test. Results are given as mean ± S.D. or percentages. The test of Hardy–Weinberg equilibrium and comparison of genotype and allele frequencies in the T2D subjects and controls have been done using the χ^2-test. To assess the extent to which the various genotypes were associated with T2D, ORs and 95% confidence intervals (CI) were estimated by logistic regression analysis. The p-values less then 0.05 were considered significant.

Results

All the case and controls were matched in age and gender. The frequency of TT genotype (mutant homozygote) in diabetic and control groups were 0 and 1 person respectively. The number of CC genotype (RR: normal homozygote) was 62 in case and 53 for control groups. The CT genotype (RW: Heterozygote) also was 63 and 71 for diabetic and non-diabetic participants, correspondingly.

The frequency of allele W (CT+TT) was 31 in diabetic patients and 36 for controls. In addition, allele R (CC+CT) frequency was 89 and 94 in controls and cases, respectively.

The polymorphic marker of SLC30A8 gene (rs13266634), which was assayed in this study, did not fallow the Hardy–Weinberg equilibrium in our study population. Gene allelic frequency showed no significant difference between two diabetic and control

Table 2. Association of the marker rs13266634 of SLC30A8 with metabolic characteristics in type 2 diabetic patients and non-diabetic controls

Characteristics	T2D(125)			Controls(125)		
	RR	RW	p	RR	RW	p
Body mass index, kg/m2	24.24±3.26	23.71±3.35	0.37	23.78±3.58	24.51±3.87	0.28
Fasting blood glucose,mg/dl	98.91±18.55	96.49±14.24	0.41	82.71±10.83	85.87±11.36	0.12
2 hr blood sugar,mg/dl	139.82±17.84	135.23±12.89	0.10	127.75±11.41	129.40±10.50	0.40
Age,years	54.64±9.19	56.47±8.62	0.25	55.26±8.41	50.52±7.80	0.002
Gender(male/female)	28/34	36/27	0.18	27/26	32/38	0.49

groups ($p<0.001$) following to calculating the allelic frequency based on hardy-weinberg equilibrium and χ^2-test. A significant difference ($p<0.05$) was detected in FBS, BS 2 hr, age, education and income status between the two diabetic and control groups [Table 1], however no meaningful association was observed between the polymorphism used in the present study and increased or decreased risk of diabetes mellitus type 2 in Azeri population of Iran (P=0.31). In genotype assaying, data analysis showed that TT genotype has no role in diabetes type 2 risk (P=0.33). In addition, we found that there was no significant discrepancy ($p>0.05$) among C/C and C/T genotypes in FBS, BMI and age mean [Table 2]. Moreover, based on findings demonstrated in Table 2, we couldn't find any association between different SLC30A8 gene genotypes and gender. (P=0.527). Our findings about t-test of means of BS 2hr in two genotype groups, C/C and C/T, also couldn't help us to find an association [Table 2].

Discussion

SLC30A8 gene encodes for a protein called Zn-T8 that exclusively expressed in pancreatic β cells. This transporter has important role in co-crystalization of insulin with zinc ions and storage of insulin in specialized vesicles until appropriate time for secretion. Different studies in European countries have shown that this gene is the most common and important gene which is changed in T2DM [21]. Non-synonymous Arg325Trp polymorphism is the most common variant of this gene that occurs at COOH terminal of protein. How this polymorphism affects Zn-T8 function, remains unknown, although some mechanisms have been suggested. For example amino acid argenin, interrupts the recognition site of protein kinase A and C and leads to lower level of phosphorylation and activity of Zinc transporter 8. Another possibility explains that this variant affects post translational modification of this protein [20].

Study of this polymorphism in T2DM, was started beside a set of other recent suspicious genes, almost in frame of GWA studies. Although most of the studies that have been done about the R325W variant of SLC30A8 gene confirm its role in decreasing risk of T2DM, it seems that the risk is different for various ethnicities. In this study we couldn't find any association between mentioned allele and its genotypes with T2DM risk. In addition no significant correlation between age, sex, FBS, BS 2 hr, BMI and CC, CT and TT genotypes was detected in case and control groups. The same results have been previously reported by other researchers about African American, Boston, Indian Asian, Moroccan and Israel Ashkenazi population [13,22-24]. However in other investigations, the results were different. For example, in a Germany assay, in spite of no correlation between risk of T2DM and SLC30A8 gene polymorphism, researchers could find meaningful association between genotypes and BMI [18].

Some other similar studies in Finnish, Russian, UK, Chinese, Indian, Asian, Canadian, Japanese and Austrian population of French study, have found significant association between this polymorphism and T2DM [7,11,22,12,13,25-29]. Moreover in assay of correlation between genotypes and metabolic and anthropometric measures, Indian population could show only association with HOMA-IR (HOMA-Insulin Resistance), an index of fasting plasma insulin level [28]. In a Russian study, correlation of RR homozygote genotype in both diabetic and control groups was significant only in BS 2 hr parameter [29] while Chinese population shows meaningful correlation between genotypes and Age, BMI, FBS and BS 2 hr unlike Sex, plasma insulin and glucose [27].

Interestingly, a Swedish study, showed that T allele cause progression from normal glucose tolerance state to T2DM in future, and unlike other assays, their investigators concluded that T allele was associated with improper β cell function [30].

These controversies specifically in African American population may be due to different genetic background and in European derived population due to haplotype obstacle in structure and allelic heterogeneity [13].

In the present study we determined significant differences in BMI, Age, gender, FBS, BS 2hr, education and economic statues between diabetic and control groups. The education and economic status have not been considered in the previous reported studies. Another potential points of our study is case-control designing. A number of studies have been carried out in cross-sectional format that have less priority to case-control studies [20,31].

Comparing the various studies investigating the correlation of R325W polymorphisms and T2D showed different results for different ethnicities, however other factors may have been involved in the results obtained in our study including small sample size or facing low number of TT homozygote genotype which, emphasizes further investigation on the subject using greater number of samples or other sub-populations of Iran including arabs, baluchs, torkmans.

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