Brief Report

Association of KCNJ11 (E23K) gene polymorphism with susceptibility to type 2 diabetes in Iranian patients

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Abstract

Background: Type 2 diabetes (T2D) is a multifactorial disease with susceptibility of several genes that are related to T2D. Insulin secretion pathway starts with potassium channels in pancreatic beta cells. KCNJ11 gene encodes ATP-sensitive potassium channel subunits. Some studies suggested that KCNJ11 (E23K) mutation increases the risk of T2D. Therefore, present study was designed to investigate the association between E23K polymorphism of KCNJ11 gene and type 2 diabetes mellitus (T2DM) in the Iranian population.

Materials and Methods: The type of study was case-control and 40 unrelated subjects, including 20 healthy controls and 20 diabetic patients were recruited (diagnosed based on American Diabetes Association criteria). Blood samples were used for isolation of genomic deoxyribonucleic acid (DNA). Having extracted the genomic DNA from human blood leukocytes by means of High Pure PCR Template Preparation Kit, PCR-restriction fragment length polymorphism method was used to detect KCNJ11 E23K gene polymorphism. *BanII* restriction enzyme was used for digestion. Data were analyzed using Chi-square or Fisher exact test or independent *t*-test, as appropriate. $P < 0.05$ was considered.

Results: We found that the carrier homozygous for KK genotype are susceptible to T2D (0.049) and in patients the frequency of K allele was higher than control subjects (0.048).

Conclusion: The present study suggests that KCNJ11 (E23K) gene polymorphism is associated with T2DM.

Key Words: Iranian population, KCNJ11, polymorphism, potassium channel, restriction fragment length polymorphism, type 2 diabetes

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INTRODUCTION

Type 2 diabetes (T2D) is a multifactorial disease that the body cannot control the glucose homeostasis and in many cases this disease is resulted by deficiency of insulin secretion and insulin activity. In general, T2D diagnosed in adults and is related to obesity, life style, age, family history and genetics. Several genes have been reported to be related of T2D susceptibility.[1,2] Prevalence of T2D has been increased in the last

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decades and relatively 250 million people are suffering from this disease world-wide. T2D is seventh reason of lethality in America.[2]

Insulin secretion pathway starts with inhibition of ATP sensitive potassium channel (KATP) channels by glucose and then by depolarization of the β-cell membrane, influx of calcium ions increased and consequently intracellular calcium stimulates the exocytosis of granules containing insulin. This channel consists of 2 subunits, region of sulfonylurea receptor and K^+ subunit (Kir 6.2). ABCC8 and KCNJ11 genes encoding these subunits respectively.[3]

KCNJ11 gene located on chromosome 11 with 4 Kb, encoding a protein with 390 amino acids. Mutation in KCNJ11 can significantly influence KATP channel activity.[4] Several mutations have been reported for KCNJ11 that two of them related to increasing risk of T2D: rs5219 for E23K mutation and rs5215 for I337V mutation. Direct effects of polymorphisms for ABCC8 have not been demonstrated.[5] Investigations have indicated substitution of glutamate (E) to lysine (K) amino acid in codon 23 of KCNJ11 gene being responsible for sensitivity reduction of KATP thus the channel is open for more time and insulin secretion is inhibited.[6,7] Therefore some studies showed correlation between E23K mutation with T2D.[8-10] Several studies showed KK homozygote genotype has most relation with T2D in Caucasian population.[3,11,12] Many studies have shown EK heterozygous have great incidence in patients and healthy population.[7,13,14] However frequency of EK in Ragia *et al*. study has not been greater than others.[15]

Therefore, evidences suggest that E23K polymorphism could be responsible for T2D. The genetic background for KCNJ11 gene for T2D has not been investigated in Iran and therefore the current study was performed to evaluate the incidence of E23K polymorphism and associations between this genetic variation and diabetes susceptibility in Iranian population.

MATERIALS AND METHODS

Subject

The type of study was case-control and was performed on 40 unrelated people that consisted of 20 healthy control and 20 diabetic patients. The subjects were recruited from Sedighe Tahere Research Center (Isfahan, Iran). Criteria for type 2 diabetes mellitus (T2DM) are based on clinical diagnoses (presence of polyuria, polydipsia and polyphagia) and were confirmed by American Diabetes Association criteria.[16] T2DM was determined by a fasting plasma glucose level of more than 126 mg/dl after a minimum fast of 12 h or a 2-h post glucose level (2-h oral glucose tolerance test) of more than 200 mg/dl or an hemoglobin A1c (HbA1c) level of >7%. Control individuals were diagnosed by a fasting plasma glucose of <100 mg/dl or a 2-h glucose of <140 mg/dl or an HbA1c level of <6.4%. Individuals with impaired glucose tolerance and positive family history of T2D were excluded from the study for control group. In order to eliminate interferences with biological variables people with pervious diagnosis of type 1 diabetes, malignity, systematic inflammation and endocrine diseases, impaired renal and hepatic function, those receiving hypercholesterolemia, hypertension or corticosteroids medications were excluded from the study. All participants signed a written consent before their enrolment for the study. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences.

Biochemical measurements

Chemical and biochemical characteristics were performed in Sedighe Tahere Research Center. Cholesterol, triglycerides, high-density lipoprotein (HDL) and plasma glucose were measured by standard enzymatic assays. Low-density lipoprotein (LDL) cholesterol was derived using Friedewald equation.[17] HbA1c was measured by means of ion-exchange high performance liquid chromatography.

Deoxyribonucleic acid analysis

Whole blood sample was used for isolation of genomic DNA. High Pure PCR Template Preparation Kit (Roche, Germany) was employed for extracting genomic DNA from human blood leukocytes. Polymerase chain reaction (PCR) restriction fragment length polymorphism method was used for detecting KCNJ11 E23K gene polymorphism. The sequences of forward and reverse primers were: 5'-GACTCTGCAGTGAGGCCCTA-3' and 5' ACGTTGCAGTTGCCTTTCTT-3', respectively. PCR amplification was performed using the following reagents: 1X PCR reaction buffer (16 mmol/l (NH4) $2SO_4$, 67 mmol/l TrisHCL and pH 8.8), 10 pmoles of each primer, 2 mmol/l $MgCl_2$, 200 μmol/l of each deoxyribonucleotide triphosphates, 100 ng of genomic DNA, 1 U Taq polymerase (Bioron, Germany) and 0.56 mmol/l DMSO. The final PCR reaction was 25 μl and PCR cycles were as follows: Initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 60 s, annealing at 60°C for 30 s and extension at 72°C for 60 s and a final extension 72°C for 9 min. The PCR amplification was confirmed by gel electrophoresis (8% acrylamide gel and Ethidium bromide staining). For digestion 8 μl of PCR product (210 bp) was digested by 3U of

a restriction enzyme *BanII* (New England, BioLabs. England) at 37°C for 1 h in 10 µl reaction mixture that contained 1X NE buffer (50 mmol/l potassium acetat 2, 20 mmol/l Tris-acetate, 10 mmol/l magnesium acetate, 1 mmol/l DDT and pH 7.9). The digestion products were analyzed by gel electrophoresis (12% polyacrylamide gel and ethidium bromide staining) with restriction pattern.

Statistical analysis

Statistical analyzes were performed using the SPSS version 21 for Windows (SPSS Inc., Chicago, IL, USA). Results are given as mean ± SD or percentage. Data were analyzed by Chi-square or Fisher exact test or independent *t*-test, as appropriate. $P < 0.05$ was considered to be significant. Chi-square test was used to test for Hardy–Weinberg equilibrium and comparison of genotype and allele frequencies in the diabetic and non-diabetic subjects [Table 1].

RESULTS

Clinical and biochemical characteristics of participants are summarized in Table 2. Statistically significant differences were observed for HDL, serum triglycerides and diabetes criteria between control and T2D patients. There were no statistically significant difference between control and T2D patient for sex, age, blood pressure, total cholesterol, LDL cholesterol and serum creatinine [Table 2].

Table 1: Hardy-Weinberg equilibrium for comparison of genotype and allele frequencies in the case and control subjects

Groups	Genotype			χ^2 value
	EE	EK	KK	
Case	3	10	7	0.03
Control	5	15	0	7.2
Total	8	25	7	2.52

χ^2 value<3.84 shows the population is in Hardy-Weinberg equilibrium

Table 2: Clinical and biochemical characteristics of type 2 diabetic and non-diabetic subjects

Characteristics	Non-diabetic subjects (*n*=20)	Type 2 diabetic subjects (*n*=20)	*P* value
Sex*(men/women)	12/8	9/11	0.264
Age (year)	49.4±5.4	49.6±7.5	0.943
BMI (kg/m^2)	24.2±2.0	27.8±3.8	0.001
Systolic blood pressure (mmHg)	121.4±8.8	123.9±19.3	0.593
Diastolic blood pressure (mmHg)	70.0±7.7	76.0±13.0	0.085
Fasting plasma glucose (mg/dl)	96.48±12.08	185.45±42.89	<0.001
2-h plasma glucose (mg/dl)	131.95±10.19	284.10±62.95	<0.001
HbA1c (%)	5.3±0.2	8.4±1.2	<0.001
Total cholesterol (mg/dl)	173.9±9.3	183.8±41.2	0.303
HDL cholesterol (mg/dl)	52.2±7.3	40.3±10.4	<0.001
LDL cholesterol (mg/dl)	98.57±7.8	88.5±37.9	0.240
Serum triglycerides (mg/dl)	112.0±16.5	213.1±95.3	<0.001
Serum creatinine (mg/dl)	0.8±0.1	0.9±0.2	0.574

Data are expressed as mean±SD.*P values refer to independent *t*-test and Fisher exact test. LDL: Low-density lipoprotein, HDL: High-density lipoprotein, HbA1c: Hemoglobin A1c, SD: Standard deviation, BMI: Body mass index

PCR amplification of KCNJ11 gene was performed and produced a 210 bp DNA band [Figure 1]. This product digested and gave different patterns as follows: E: 150 bp, 32 bp and 28 bp; K: 178 bp, 32 bp and EK: 178 bp, 150 bp, 32 bp and 28 bp [Figure 2].

The association between E23K polymorphism and T2D risk was analyzed. As shown in Table 3, carrier homozygous for KK genotype is susceptible to T2D and in patients the frequency of K allele was higher than control subjects. In addition, heterozygous carriers for EK are more in non-diabetic patients ($P = 0.049$).

Association of different genetic models with control diabetes criteria in T2D patients according to E23K KCNJ11 genotype was analyzed in Table 4. According to the results there was significant difference ($P = 0.005$) between KK and combined genotype (EK + EE) for 2-h plasma glucose. Furthermore, a significant difference was shown between HDL of non-diabetic subject and T2D patients ($P = 0.017$).

DISCUSSION

Prevalence of T2D has increased in the last decades and some genes have been associated with T2D.[2] In our study, KCNJ11 (rs5219) gene was analyzed for investigation of the association between E23K polymorphism with prevalence of T2D. As you can

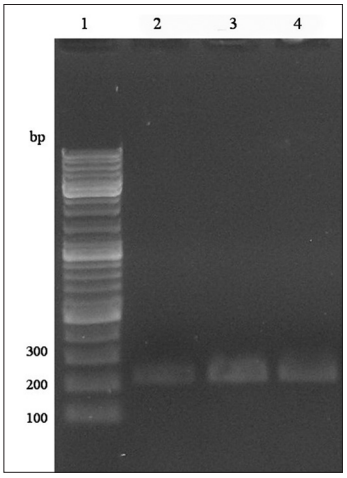

Figure 1: Confirmation polymerase chain reaction (PCR) amplification of KCNJ11 gene using gel electrophoresis. Lane 1:100 bp deoxyribonucleic acid ladder; lane 2, 3 and 4: PCR products (210 bp)

see in Table 3, statistical analysis showed people with KK homozygous genotype have more susceptibility for this disease (P = 0.004). A study in Russia also found KK homozygous genotype had more risk for T2D (P = 0.016).[12] Moreover, we found that carriers of K allele have more risk for T2D (P = 0.048), Chistiakov *et al*. also found similar results for Russian population (P = 0.023).[12] In Li study also found E23K contributed to T2D susceptibility.[18] In a study published in 2013, Asaf *et al*. had showed the inheritance of the K allele predisposes for T2D.[19] However in Wang *et al*. study relationship between the E23K and T2D not seen in Han people in Qingdao area.[20]

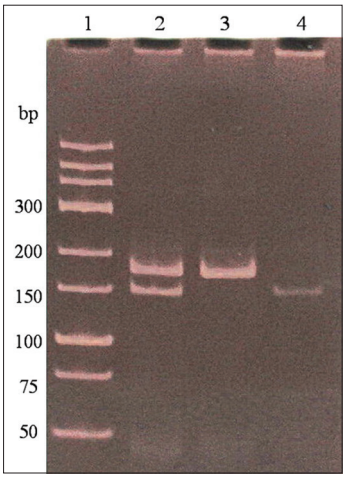

Figure 2: Polymerase chain reaction products digested by BanII restriction enzyme for the detection of E23K polymorphism of KANJ11 gene. Samples were electrophoresed using a 12% polyacrylamide gel and subsequent staining with ethidium bromide. Lane 1: Deoxyribonucleic acid ladder; lane 2: E23K heterozygotes; lane 3: K23 homozygotes; lane 4: E23 homozygotes. Small bands of 32 bp and 28 bp are not visible in this figure

Table 3: Association of KCNJ11 (E23K) with type 2 diabetes

Genotype	Control (%)	Case (%)	*P*	Odds ratio (95% CI)
EE	5 (25)	3 (15)	0.347	0.52 (0.11-2.59)
EK	15 (75)	10 (50)	0.049	2.12 (1.01-5.39)
KK	0 (0)	7 (35)	0.004	1.53 (1.11-2.12)
K allele	15 (37.5)	24 (60)	0.048	2.5 (1.01-6.14)

P value refers to Chi-square test and Fisher exact test. CI: Confident interval

We found that frequency of EK genotype is more in non-diabetic patients (P = 0.049). In U.K. prospective diabetes study frequency of EK in T2D was a little more than glucose tolerated people.[21]

Frequency of E allele in healthy people is more prevalent, thus E allele carries probably have lower risk for T2D when compared with carries of K allele (P = 0.048).

In our study, we did not observe a significant association between dominant genetic models with diabetes control criteria [Table 4]. However in recessive model, a significant correlation with HDL cholesterol was found (P = 0.017), so carriers of dominant E allele have more HDL in comparison with KK genotype (P = 0.017). In biochemical characteristics of T2D and non-diabetic people a significant association between HDL and serum triglycerides with two groups was found, so non-diabetic people have more HDL and low serum triglycerides in comparison to T2D patients. This finding could be due dyslipidemia in diabetic patients.[22] In a study by Riedel *et al*., a mechanistic link was proposed between increased body mass index and plasma free fatty acid levels with high prevalence of T2D by the KATP channel homozygous polymorphic.[23] We also found that population with KK homozygous genotype has more susceptibility to low HDL (P = 0.017).

CONCLUSION

KCNJ11 (E23K) polymorphism has relationship with susceptibility to T2D in Iranian population and carriers of K allele are more susceptible to this disease. Additional studies with larger sample size will be required to confirm this marker in Iranian population.

Table 4: Association of different genetic model with diabetes control criteria in T2D patient according to E23K KCNJ11 genotype

Genetic models	Recessive (*N*=20)			Dominant (*N*=20)		
	KK (*N*=7)	EE+EK (*N*=13)	*P*	EE (*N*=3)	EK+KK (*N*=17)	*P*
BMI (kg/m^2)	29.8±2.3	26.8±4.2	0.104	25.7±6.1	28.2±3.4	0.321
Fasting plasma glucose (mg/dl)	163.8±21.6	196.2±46.8	0.102	151.2±30.6	190.8±41.4	0.156
2-h plasma glucose (mg/dl)	232.2±50.4	309.6±50.1	0.005	313.2±18	277.2±66.6	0.376
HbA1c (%)	8.4±1.3	8.4±1.2	0.918	7.8±1.1	8.5±1.2	0.400
Total cholesterol (mg/dl)	170.1±34.8	189.5±42.5	0.301	197.2±23.2	177.9±42.5	0.473
HDL cholesterol (md/dl)	27.1±11.6	42.5±7.7	0.017	34.8±0.4	34.8±11.6	0.773
LDL cholesterol (mg/dl)	73.5±42.5	92.8±34.8	0.352	100.5±38.6	85.1±38.8	0.503
Serum triglycerides (mg/dl)	194.9±88.6	212.6±97.4	0.682	301.1±88.5	194.8±79.8	0.060

Data are expressed as mean±SD. *P* values refer to independent *t*-test. LDL: Low-density lipoprotein, HDL: High-density lipoprotein, HbA1c: Hemoglobin A1c, SD: Standard deviation, BMI: Body mass index

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