Original Article

Pharmacogenomics of Sulfonylureas Response in Relation to rs7754840 Polymorphisms in Cyclin-Dependent Kinase 5 Regulatory Subunit-associated Protein 1-like (CDKAL1) Gene in Iranian Type 2 Diabetes Patients

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Abstract

Background: Sulfonylureas are important drugs of choice for treatment of type 2 diabetes mellitus (T2DM). It is suggested that differential response to sulfonylureas from T2DM patients is under influence of single nucleotide polymorphisms in some of the target genes. In spite of favorable therapeutic effects, sulfonylureas are associated with some adverse side effects such as microvascular complications and stroke, especially in older patients. Therefore, for T2DM patients who are getting less benefit, sulfonylureas should be avoided. Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like (CDKAL1) gene variation is reported to be associated with sulfonylureas effectiveness. Due to the inconsistency of available data regarding association of rs7754840 in CDKAL1 gene with sulfonylureas response in T2DM patients, the present study is conducted. **Materials and Methods:** Fifty-one diabetic patients sensitive to sulfonylureas and 51 patients resistant to sulfonylureas treatment were recruited to this study. After extraction of DNA from patients' peripheral blood samples, rs7754840 single-nucleotide polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism assay using *MaeII* (*Tail*) restriction enzyme. **Results:** Frequency of G allele in resistant group was more than sensitive group (71, 6% vs. 57, 8%). Regression analysis was shown significant association between GG genotype and higher risk of resistance to sulfonylureas treatment (odds ratio = 2.250 [95% confidential intervals: 1.010–5.012]; $P = 0.046$). **Conclusion:** Our data confirmed that genotypes of rs7754840 are significantly associated with sulfonylureas treatment response. rs7754840 in CDKAL1 gene in combination with other clinicopathological findings would help to move towards personalized therapy of T2DM patients.

Keywords: *Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like, glycemic control, single nucleotide polymorphism, sulfonylureas, type 2 diabetes*

Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disease that results from the interaction of genetic and environmental factors.[1] Hyperglycemia, the hallmark of T2DM, accompanying with impaired metabolism of proteins and fats, is due to the defective insulin action or insulin secretion.[2] Hyperinsulinemia, beta-cell dysfunction, and insulin resistance are the pathophysiological determinants of dysglycemia and type 2 diabetes.[3] Genetics play a significant role in susceptibility to diabetes and association of >40 genes with T2DM has been proved by genome-wide association (GWA) studies.[4] Lifestyle changes and metformin monotherapy are usually considered for T2DM patients as therapeutic steps. Sulfonylureas are prescribed as second-line therapy during the progression of disease or failure of getting satisfactory effects with metformin alone.[5,6] Sulfonylureas induce secretion of insulin in pancreatic beta-cells by attachment to the sulfonylurea receptors 1 subunit of ATP-sensitive potassium (KATP) channel. This interaction makes KATP channel closed leading to prevent the outflow of potassium. It induces calcium channel opening and elevation of intracellular calcium which in turn results in insulin secretion from the beta cells.[7,8] Although sulfonylureas are common affordable options for treatment of type 2 diabetes,[9] there are some concerns about several reported adverse side effects such as weight gain,

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increased cardiovascular complications, risk of stroke, and hypoglycemia.[10-12]

Substantial interindividual differences are shown in glucose-lowering effect of sulfonylureas.[13] Cyclin-dependent kinase 5 (CDK5) regulatory subunit- associated protein 1-like (CDKAL1) is one of the genes whose strong association with T2DM has been reported in several GWA studies.[14-18] CDKAL1 gene in 6p22.3 produces a 65 KDa protein with unknown function that is similar to CDK regulatory subunit-associated protein 1 (CDK5RAP-1) expressing in pancreatic beta-cells. CDK5RAP1 protein inhibits CDK5 activity by binding to P35 and activator of CDK5. CDK5 inhibits insulin secretion in response to glucose and decreased insulin gene expression, so it is associated with the pancreatic beta-cell malfunction and susceptibility to T2DM. CDKAL1 plays a role in beta-cell function through inhibition of CDK5.[19-22] Carrying risk variants of CDKAL1 may attenuate normal functioning of CDKAL1 resulting in impaired insulin secretion.[16,23] Many studies have shown that rs7754840 in CDKAL1 gene is associated with risk of type 2 diabetes.[24] CC homozygotes rs7754840 was related to decreased insulin secretion in the first phase and not in the second phase during the hyperglycemia clamps and glucose tolerance tests.[25,26] Furthermore, the relationship between this single-nucleotide polymorphism (SNP) and defects in insulin secretion and processing are reported.[26-28] A replication study showed that the C allele and CC genotype of rs7754840 were associated with increased risk of T2DM in Iranian population.[29] Gene variants in CDKAL1 were also reported to be associated with sulfonylureas treatment outcome in T2DM patients.[13,30,31] Association of SNP rs7754840 in CDKAL1 gene with sulfonylurea treatment effect is not yet determined in Iranian population. Therefore, the aim of this pharmacogenetic study was to evaluate relationship of rs7754840 genotypes in CDKAL1 gene with sulfonylureas treatment in T2DM patients sensitive and resistant to this drug.

Materials and Methods

Diabetic patients selected on the basis of the World Health Organization criteria (fasting plasma glucose 0.7 mmol/l and/or 2 h plasma glucose >11.1). Totally, 102 T2DM patients were recruited to this study (51 sensitive and 51 resistant diabetic patients to treatment with sulfonylureas). Resistant T2DM patients were selected on the basis of having combined therapy of metformin and sulfonylurea for 12 months, but their HbA1c failed to maintain <7.0%. Sensitive patients were selected among those who have maintained HbA1c<7.0% during 12 months combined therapy. Patients who started insulin therapy were excluded from the study. Informed consent signed by all participants based on a standard questionnaire was approved by the Ethics Committee of the Isfahan University of Medical Sciences. Anthropometric characteristics of the participants were collected through a structured questionnaire.

Genotyping of single-nucleotide polymorphism rs7754840 (C/G) polymorphism

Peripheral blood samples collected from participants' genomic DNA were extracted from blood samples by Genetbio DNA extraction kit (Korea) according to the manufacturer's instructions. Genotyping was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. PCR was performed by CDKAL1-specific primers: forward TCTTGGAGTAGTCACCTGGTCA and reverse AAAAATCCTCGCAACAACAGA. PCR thermal cycling and durations of PCR cycles were as follows: initial denaturation at 95°C for 6 min, followed by 34 cycles at 94°C, 58°C, and 72°C all for 50 s. A final extension of 5 min was applied after the completion of all 34 cycles and hold at 4°C. Subsequently, products of PCR were digested by *MaeII* (*Tail*) restriction enzyme for 12 h at 65°C and analyzed on 2% agarose. After digestion, the presence of two bands of 495 bp and 286 bp was indicative of CC genotype, but a single band of 781 bp represented GG genotype. In heterozygote genotypes (GC), three bands of 495 bp, 286 bp, and 781 bp became visible on the gel. Confirmation of RFLP-based genotyping was done by direct sequencing of some 10% of randomly selected samples.

Statistical analyses were accomplished by SPSS for windows software (Version 22.0; SPSS, Chicago IL, USA). Chi-square test was used to evaluate allele and genotype frequencies for Hardy–Weinberg equilibrium. For calculation of distributions and risk allele/genotype-specific odds ratios (OR) (OR, 95% confidential intervals [CI]) and analogous *P* values after adjustment for gender, age, and body mass index (BMI) as covariates, logistic regression analyses were performed. Continuous variables were introduced as mean ± standard deviation and compared between sensitive and resistant groups using independent student's *t*-test.

Results

Clinical and biochemical characteristics of the participants

Baseline clinical and biochemical characteristics of the patients are displayed in Table 1. The mean age and BMI level were 57.21 ± 9.92 years and 25.79 ± 3.19 kg/m² for the sensitive patients and 55.68 ± 9.06 years and 25.63 ± 3.70 kg/m² for the resistant patients, respectively. No significant differences were shown in age and BMI among the two groups. Mean changes of HbA1c in sensitive and resistant patients, 4 times every 3 months during the test period of 12 months, are shown in Table 1.

Association of rs7754840 polymorphism with drug response in type 2 diabetes mellitus patients

Genotype and allele frequencies of the sensitive and resistant diabetic patients are shown in Table 2. Frequencies of the CC, GG, and GC genotypes of rs7754840 were 17.6, 33.3, and 49.0% in sensitive group and 9.8, 52.9, and 37.3% in resistant group, respectively. Between these three genotypes of the rs7754840 in CDKAL1 gene, GG was found meaningfully associated with increased risk of resistance phenotype to sulfonylureas (OR = 2.250 [95% CI: 1.010–5.012]; P = 0.046). The frequency of G allele in resistant patients (71.6%) was more than sensitive patient group (57.8%); therefore, this allele would be risk allele for conferring resistance to sulfonylureas (OR = 1.835 (95% CI: 1.024–3.286); P = 0.040). Compared with the GG genotype (as resistance genotype), CC + GC genotypes determined to be associated with sensitive phenotype and more responsive to sulfonylureas treatment.

Discussion

Diabetes is a disease that if not diagnosed early, not treated quickly, and properly managed, becomes serious health disaster.[1] The importance of good glycemic control in the prevention of microvascular complications of diabetes is generally accepted.[2,32,33] Differences in response to sulfonylureas in diabetic patients make a flaw in management of diabetes complications. Therefore, early identification of T2DM patients for whom sulfonylureas are less beneficial and likely experience adverse events would much help in patients managements.[34,35] Patients who are not getting benefit from sulfonylureas consumption may unnecessary exposed to unfavorable side effects of the drug such as increased risk of cardiovascular disease, stroke, and weight gain without ideal glycemia control.[9,36-39]

Here, we showed that carriers of G allele and GG genotypes of the rs7754840 SNP in CDKAL1 gene have an increased risk of resistant phenotype to sulfonylureas treatment compared with GC + CC genotypes. To the best of our knowledge, this is the first study in Iranian population that provides association between genotypes of rs7754840 SNP and sulfonylureas treatment outcome in T2DM patients.

In mice lacking the CDKAL1 gene, the decline in first-phase insulin secretion and defects in mitochondrial ATP production has been observed.[19] CDKAL1 by facilitating the production of mitochondrial ATP would make KATP channel to be active as well as an increase in intracellular calcium by activating calcium channels that are consequently lead to insulin exocytosis.[40] This function of CDKAL1 can explain the relationship between gene variants of CDKAL1 and response to sulfonylureas.[13,30] Associations of some variants of CDKAL1 gene with therapeutic response to sulfonylureas are reported in some studies. In Caucasians, the study of rs7756992 polymorphism of CDKAL1 gene reported a prominent higher reduction in HbA1c at 6 months in heterozygotes and homozygotes of the risk allele A carriers.[13] Haplogenotypes of CDKAL1 that have the risk of T2DM were associated with decreased response to nonsulfonylureas and sulfonylureas agonists of pancreatic KATP channel in Russian population. According to this study, in sulfonylureas consumers, internal circulating insulin in postprandial period was much better in C allele carrier of three SNPs of CDKAL1 gene (allele C of rs10946398, allele C of rs7754840, and allele G of rs7756992).[30] In rs10811661 polymorphism in the CDKN2A/2B gene, which also has similar function as CDKAL1, CC risk allele homozygotes showed significant reductions in free plasma glucose in response to sulfonylureas.[31] Inconsistent with our study, in two

Table 1: Demographic characteristics of type 2 diabetes mellitus patients with different sulfonylureas treatment response

Variables	Mean±SD		*P*
	Resistant patients	Sensitive patients	
Age	55.68±9.06	57.21±9.92	0.418
BMI	25.63±3.19	25.79±0.815	0.815
HbA1c 1	9.48±1.52	7.27±1.96	>0.005
HbA1c 2	8.86±1.85	6.69±1.18	>0.005
HbA1c 3	8.75±1.68	6.32±0.92	>0.005
HbA1c 4	9.03±1.72	6.18±0.85	>0.005

Data are presented as mean ± SD. BMI: Body mass index, HbA1c: Hemoglobin A1c, SD: Standard deviation

Table 2: Allele and genotype distribution of rs7754840 single-nucleotide polymorphism in type 2 diabetes mellitus patients with sensitive and resistant response toward sulfonylureas treatment

Variables	Resistant's patients, *n* (%)	Sensitive's patients, *n* (%)	Resistant or sensitive Allele/genotype	OR (95% CI)	*P*
Allele frequency					
G	73 (71.6)	59 (57.8)	G	1.835 (1.024-3.286)	0.040
C	29 (28.4)	43 (42.2)	C		0.040
Genotype frequency					
GG	27 (52.9)	17 (33.3)	GG	2.250 (1.010-5.012)	0.046
CC	5 (9.8)	9 (17.6)	CC		0.046
GC	19 (37.3)	25 (49.0)	GC		0.046

OR: Odds ratio, CI: Confidence interval

studies of Japanese and Chinese population, no significant differences were observed in response to sulfonylureas between genotypes of rs7754840 of CDKAL1.[41,42] This inconsistency is probably because of the genetic differences in various populations.

Our study is different with other similar studies[13,30,31,41,42] in the way that we genotyped two distinguished groups of sensitive and resistant patients for sulfonylureas treatment that were homogenous in number, age, and BMI. Furthermore, all of the T2DM patients recruited to this study started their drug therapy with metformin monotherapy and later due to the failure of metformin to manage acceptable glycaemia level switched to a drug from sulfonylureas family. Patients were divided into sensitive and resistant groups, based on changes in the HbA1c over a period of 12-month therapy during which every 3 months laboratory data were collected.

Conclusion

Our study proved that GG genotype of the SNP rs7754840 is associated with high risk of sulfonylureas-resistance phenotype compared to CC + GC genotypes in Iranian population. Studies of this sort would facilitate to find a cohort of suitable biomarkers for drug efficiency evaluation in T2DM patients. Personalized prescription of glycaemia control drugs would make diabetes management more successful. Prior genetic testing using suitable biomarkers make personalized prescription possible. Right drug administration means less unfavorable side effects and fast glycemic control and therefore preventing hyperglycemia-related complications in T2DM patients.

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Conflicts of interest

There are no conflicts of interest.

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