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RELATIONSHIP BETWEEN PPARGC1A GENE POLYMORPHISMS WITH THE INCREASED RISK OF CORONARY ARTERY DISEASE AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS IN IRAN

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Abstract

Background. Type 2 diabetes (T2D) increases the risk of coronary artery disease (CAD) in patients with type 2 diabetes compared with nondiabetic subjects. Several genetic variants are considered as risk factors for CAD, including those implicated in dyslipidaemia and oxidative stress. The PPARGC1A gene is considered as a key regulator of pathophysiological processes contributing to CAD.

Aim. We investigated whether the Gly482Ser polymorphism (rs8192678) increased susceptibility to CAD in Iranian population and whether it was associated with clinical and metabolic parameters.

Patients and methods. A total of 290 subjects including 149 CAD patients with a history of diabetes and 149 controls were included in our study. The Gly482Ser polymorphism was genotyped using ARMS-PCR method. Based on the type of variables, by the use of SPSS software (Statistical Package for Social Sciences Inc., Chicago, IL, USA) statistical analyses were performed.

Results. We found a significant difference in the Gly482Ser substitution between the case and control subjects in Iranian population. However, no significant association was observed between Gly482Ser genotypes and physiologic variables.

Conclusion. This gene polymorphism PPARGC1A Gly482Ser may be a potential marker for increased risk of CAD in diabetic patients in clinical treatment and diagnosis in the Iranian population.

Key words: Coronary artery disease, PPARGC1A gene, CAD susceptibility.

INTRODUCTION

One of the cardiovascular complications of diabetes mellitus is coronary artery disease (CAD) (1). Type 2 diabetes increases the risk of CAD at least by two- to three fold in patients with type 2 diabetes compared with nondiabetic subjects (2). In addition to environmental factors, such as lifestyle, obesity, sedentary life and smoking, several genetic variants are considered as risk factors for CAD, including those implicated in dyslipidaemia and oxidative stress (1, 3, 4). Peroxisome proliferator-activated receptor (PPAR) is a key transcription factor in adipocyte differentiation (5). Peroxisome proliferator-activated receptor-G coactivator-1 alpha (PPARGC1A) is a transcriptional co-activator of PPAR (6, 7). The PPARGC1A gene encoding for PGC-1α is a strong biological candidate for cardiovascular and metabolic disease. It is also considered as a key regulator of pathophysiological processes contributing to CAD (8, 9). PGC-1 interaction with PPAR-α regulates mitochondrial fatty acid oxidation enzyme gene expression in the heart, brown adipose tissue and liver (10). PGC-1α has also been considered to up-regulate glucose transporter 4 and, as a result, increase glucose uptake in the muscle (11). In addition, PGC-1α is implicated in hepatic gluconeogenesis by increasing gene transcription of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (12). The well established role of PGC-1 as a critical regulator for adaptive cellular energy metabolism, vascular stasis, oxidative stress and adipogenesis has prompted many scientists investigation on associations between

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PPARGC1A variants and a range of seemingly plausibly disease traits (8). Previous studies have reported that PGC-1α also regulates lipid metabolism (13). Given the critical role of PGC-1α assumes in oxidative stress and lipid and glucose oxidation, it may be involved in the initiation and development of atherosclerosis and diabetes (9).

Several polymorphisms within the PPARGC1A gene have been reported to be associated with type 2 diabetes mellitus, obesity and hypertension, although the results are controversial (14-16). Among these the most widely studied PPARGC1A variant is the Gly482Ser (+1564G/A) polymorphism. Since the initial report of association between this variant and type 2 diabetes, many studies have reported associations with diabetic complications (17-19).

In the present study, we investigated whether the Gly482Ser polymorphism (rs8192678) increased susceptibility to CAD in Iranian population and whether it was associated with clinical and metabolic parameters.

MATERIALS AND METHODS

A total of 290 subjects including 149 CAD patients with a history of diabetes and 149 controls were included in our study. CAD was confirmed by coronary angiography. Exclusion criteria for CAD patients were presence of diabetes mellitus, cardiomyopathy, chronic obstructive pulmonary disease, collagenoses, chronic liver disease, infections or inflammatory diseases, thyroid disease, drugs abuse, and chronic alcohol abuse. The selection criteria also included no history of coronary intervention, organ transplantation, or renal hemodialysis, and no history of MI and stroke. For controls, additional exclusion criteria were symptoms of CAD and familial history of cardiovascular disorders. In this study, traditional risk factors were characterized on the basis of the European Society of Cardiology standards and recommendations (20). Current cigarette smoking was defined as a daily intake of more than five cigarettes. Nonsmokers included former smokers who had quit smoking at least 1 year before the study. Hypertension was defined as systolic pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg in at least two separate measurements. Overweight was defined as BMI greater than 25 kg/m^2 and obesity BMI ≥30 kg/m^2. All subjects had plasma glucose ≥ 6.95 mmol/L in the fasting state. Hypercholesterolemia was considered present if total cholesterol serum levels were ≥5 mmol/L or if the subject was undergoing a treatment with cholesterol-lowering drug. The characteristics of the population investigated are given in Table 1.

Informed consent was obtained from all study participants, and the study was approved by the institutional ethics committee. All examined individuals were instructed to fast for at least 12 h before blood collection. Total serum cholesterol, HDL cholesterol, and triglycerides were measured by enzymatic methods (commercial Analco Kit, Pland). Low-density lipoprotein cholesterol levels were calculated according to the Friedewald formula (21).

Genomic DNA was extracted from peripheral blood lymphocytes using Bioneer DNA isolation kit. Sequencing was performed using the Big Dye Terminator chemistry on an automated DNA sequencer (Model 3700; Applied Biosystems). The Gly482Ser polymorphism was genotyped using Amplification refractory mutation system (ARMS) (ARMS-PCR) method. The ARMS technique, a simple PCR-based method, uses the amplification arrest caused by non-complementary nucleotide(s) which are located at 3' end of the primer. In the present study we used a mutation-specific primer, resulting in the formation of an additional product only in the presence of the A allele. Three primers were used: one forward primer 5'- GACGAAGCAGACAAGACCG -3' and two reverse primers 5'- GACGAAGCAGACAAGACCA-3' (mutation specific) and 5'– AGAGTTAAAAGAAGAACAAGAAGGAG -3' (control).

Thermal cycling was carried out as follows: 95°C

Table 1. Characteristics of participants in this study

Characteristics	CAD Subjects	Control Subjects	P value
N (m/f)	72/73	71/74	0.831
Mean Age (yrs)	9 ±53.9	51.3 ± 10	0.423
Mean Weight (kg)	89.2 ±3.8	86.3 ± 4.4	0.577
Systolic BP (mmHg)	139.5 ±24.8	131.2 ±19.8	0.001
Diastolic BP (mmHg)	80.2 ±11.8	83.6 ±9.4	0.002
Total cholesterol (mg/dL)	204.1 ±28.4	198.8 ±34.1	0.001
BMI (kg/m^2)	29.4 ± 4.5	24.6 ± 2.6	0.756
FBS (mg/dL)	82.1 ± 2.3	89.6 ± 9.1	0.572
2h plasma glucose (mmol/L)	9.1 ± 3.1	5.4 ± 7.4	0.012

All data expressed as a mean (±SD), CAD: coronary artery disease; BP: blood pressure; BMI: Body mass index; FBS: Fetal blood sampling.

for 5 minutes, then 30 cycles of 95°C for 30 seconds, 61.2°C (for H63D mutation) and 57.2°C (for C282Y mutation) for 30 seconds, and 72°C for 30 seconds. This was followed by incubation at 72°C for 5 minutes. The PCR amplification was tested by running 10 μL of the product on a 2% agarose gel.

To assure that the genotyping was of adequate quality, sequencing was performed.

Based on the type of variables, by the use of SPSS software (Statistical Package for Social Sciences Inc., Chicago, IL, USA) statistical analyses were performed. Categorical variables including the frequencies of genotypes and alleles were compared using the χ^2 test and continuous variables by Student's t-test. The odds ratio (OR) and 95% confidence intervals (CIs) were calculated to estimate the strength of the association between polymorphism genotype alleles and cases and controls. Data are presented as mean ± standard deviation for continuous variables and p values of <0.05 were considered statistically significant.

RESULTS

The PCR products were resolved by 2% agarose gel. The genotype and allele frequencies of the Gly482Ser polymorphism are presented in Table 2. Two genotypes including AG and AA were detected, but no GG genotype was found.

Analysis of the associations between the Gly482Ser polymorphisms and physiologic variables are shown in Table 3. After genotyping, the effects of the Gly482Ser polymorphisms on quantitative and categorical variables were analyzed. The frequency of AG and AA genotypes among cases was 72% and 28%, respectively, compared with 44.4% and 55.6%, respectively among control subjects. When compared with AA genotype, the heterozygote GA genotype was associated with a significantly increased risk of CAD (p<0.01) (adjusted odds ratio (OR) = 3.21, 95% confidence interval (CI) = 1.54–6. 68).

We further analyzed the associations between Gly482Ser polymorphism and physiologic variables which are shown in Table 3.

The relationship between obesity and two genotypes was evaluated in both groups. People with a body mass index (BMI) below 27 were considered as the lean and obese subjects with BMI greater than or equal to 27 were considered as fat individuals. Data analysis in regard of BMI showed no significant differences between two genotypes in case and control groups (p=0.17).

Because PGC-1α participates in lipid metabolism, the plasma lipid levels were analyzed according to the Gly482Ser polymorphism in both groups. In total, no significant differences between GA and GG genotypes in CAD patients and controls were detected in FBS, plasma lipids levels (TC, TG, LDL-C, and HDL-C); HbA1c, micro albuminuria and creatine levels were noticed.

Table 2. Genotypic and Allelic Associations of Gly482Ser Polymorphism with CAD

Variable	Genotype		OR (95% CI)	P-value
	AA	AG		
CAD Subjects	28%	72%	6.68 (3.21)-1.54	0.001
Controls Subjects	55.6%	44.4%		

All data expressed as a mean (±SD), CAD: coronary artery disease

Table 3. Analysis of physiologic variables in control and case groups

Variable	CAD Subjects			Controls Subjects		
	AA	AG	P-value	AA	AG	P-value
N	53	92	-	81	64	-
BMI (Kg/m^2)	29.4±4.7	29.2±4.4	0.46	24.6±3.5	24.58±3.4	0.86
FBS (mg/dL)	90.2±2.2	82.1±2.3	0.17	89.6±9.1	87.4±9	0.15
Cr. (mg/dL)	1±0.3	11±0.4	0.18	1±0.3	1.1±0.4	0.52
(mg/dL) TG	110.1±2	104.2±2	0.95	76.5±1.5	71.9±1.5	0.99
Chol.	42.2±1.8	48.5 ±1.8	0.39	48±1.6	44.7±1.7	0.2
HDL-C (mg/dL)	52.4±11.9	49.7±11.9	0.28	39.7±10.3	39.7±11.8	0.96
LDL-C (mg/dL)	88.1±32.2	89.9±35.5	0.53	50.6±13.3	52.4±12	0.4
HbA1c (mg/dL)	8.3±1.9	8.5±2	0.39	7.8±1.5	7.7±1.6	0.65
Microalbumin (mg/dL)	20.7±7	20.6±6.8	0.47	6.3±1.9	6.8±2.1	0.18

All data expressed as a mean (±SD), CAD: coronary artery disease; BMI: body mass index; LDL-C: low-density lipoprotein–cholesterol; HDL-C: high-density lipoprotein–cholesterol; TG: Triglycerides; Cr: Creatine; Cholesterol: Chol.

DISCUSSION

CVD is the most prevalent cause of mortality and morbidity among diabetic patients. Adult diabetic patients present levels of mortality due to heart disease and stroke from two to four times higher than those without diabetes (22, 23). It has been stated that patients with diabetes aggregate other comorbidities such as obesity, hypertension, and dyslipidemia which also cause to elevate the risk for CVD (24).

PGC-1α is a transcriptional co-activator of PPAR and has been implicated in the regulation of genes involved in energy metabolism adipogenesis and lipid metabolism. PGC-1α facilitates PPAR-α and PPAR-γ mediated gene transcription by co-activating these nuclear receptors and is therefore implicated in controlling adipogenesis and lipid metabolism. Evidences in regard to the role in regulating gluconeogenesis and glucose uptake further links this co-activator to type 2 diabetes (6, 7). Studies in human, bovine and mouse endothelial cells show that overexpression of PGC-1α decreases ROS accumulation and apoptosis during oxidative stress and under basal conditions. It has been stated that this protective role can be further attributed to the up-regulation of the mitochondrial antioxidant defense system by PGC-1α at the transcriptional level. Moreover, as PGC-1α integrates the transcriptional network, it regulates both lipid and carbohydrate metabolism for an equivalence between energy storage and consumption (9). The most frequently studied PPARGC1A polymorphism is the Gly482Ser variant (8). Gly482Ser polymorphism may change the expression of PGC-1α and/or its interaction with other transcription factors required to regulate lipid metabolism and oxidative stress, which finally triggers the pathogenesis of CAD. Gly482Ser has been widely reported to be associated with an increased risk of type 2 diabetes in some populations (6, 9).

It is a known fact that patients with diabetes, as a result, have accelerated atherosclerosis and insulin resistance status is a risk factor for myocardial infarction (MI). Therefore, a polymorphism which is associated with type 2 diabetes or insulin resistance might also contribute to MI risk (5).

However, a thorough investigation of common PPARGC1A variants and their associations with coronary artery disease traits is yet to be reported (8). Recently, Zhang *et al*. showed that the Gly482Ser polymorphism of PGC-1 was associated with an increased risk of CAD in a Chinese population. Owing to these reported associations and the role of PPARGC1A in association with diabetic complications, in the present study, we wanted to determine whether the Gly482-Ser polymorphism is associated with susceptibility to CAD. In consistent with the report in Chinese population (16), we found a significant difference in the Gly482Ser substitution between the case and control subjects in Iranian population. Compared with the AA genotype, the heterozygote GA genotype showed a significant 73% increased risk of CAD. Moreover, our observations are in contrast with the results of Iglseder *et al.* in Austrian population. However, due to lack of the GG genotype in our study subjects, we were unable to determine the mean for CAD of this genotypic group.

Several populations have shown a relationship between the PPARD variant and higher cardiovascular risk mediated by impaired lipid metabolism and hyperlipidemia. Also high levels of PRARβ/δ expression could up-regulate the expression of ABCA1, a membrane transporter, responsible for cholesterol efflux (25). Our findings are in agreement with Nikitin *et al.* who found a potential effect of the PPARD gene on enzyme activity (26).

We performed a stratified analysis to assess the co-impacts of Gly482Ser variations with other known risk factors on CAD. There is a significant association between PPARGC1A mRNA expression and HDL-C levels. We investigated the relationship between the Gly482Ser genotype and plasma lipid levels in all participants. However, no significant association was observed between Gly482Ser genotypes and plasma lipid profiles. The reason for this lack of association could be the relatively small population size. Furthermore, lipid metabolism is influenced by several genes. Also there was no association of the Gly482Ser polymorphism with BMI, FBS, plasma lipids levels, HbA1c, micro albuminuria and creatine levels.

The results indicate that the PPARGC1A Gly482Ser polymorphisms have an impact on CAD susceptibility in the Iranian population. According to the results obtained in this research, it is recommended that persons who are diagnosed in early stages of diabetes must be tested to study PPARGC1A gene to find persons with potential diabetic retinopathy and cataract and treated properly. These variants are unlikely to interact with each other and hence contribute to the cardiovascular risk through independent and distinct mechanisms. Similar conclusions were reached by another research paper who reported a significant association between PPARGC1A gene mutations and increased risk of incident CAD in women but not in men (27).

In conclusion, these data indicate that the PPARGC1A Gly482Ser missense polymorphism in PGC-1 is associated with CAD risk in the general population in Iran. As a result it could be concluded that this gene polymorphism may be a potential marker for increased risk of CAD in diabetic patients.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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