SIRT1 gene is associated with cardiovascular disease in the Iranian population

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Abstract Background: Sirtuins (SIRT) have recently been identified as the pivotal regulators of lifespan and health. SIRT1 has protective effects against cardiovascular disease (CVD) and through its deacetylase activity it regulates numerous essential pathways including regulating blood pressure, reducing atherosclerosis, heart protection against oxidative stress and inducing cardiac cell survival and growth.

Aims: Therefore, this study was conducted to evaluate whether two genetic polymorphisms of SIRT1 rs3758391 T/C and rs369274325 G/T are associated with the risk of CVD.

Material and methods: A total of 500 Iranian subjects including 250 CVD patients and 250 healthy individuals as the control group were recruited in this case-control study. Genotyping of SIRT1 rs3758391 T/C and rs369274325 G/T polymorphisms were performed using PCR-RFLP and Tetra-ARMS PCR methods, respectively.

Results: Our findings indicated a significant difference between two groups regarding the SIRT1 rs3758391 CC genotype in both additive and recessive models. The rs3758391 CC genotype was found to be more frequent in CVD patients than in the controls (19% vs. 6%), suggesting a statistically significant difference in either of additive (CC vs. TT; OR = 3.06, P = 0.001) as well as recessive models (CC vs. TT + CT genotype; OR = 3.72, P = 0.001).

Conclusion: Our study for the first time suggests that the SIRT1 rs3758391 T/C polymorphism may confer an increased risk of CVD in both additive and recessive models, in this Iranian population.

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1. Introduction

In the last decades the worldwide population has demonstrated a mounting life expectancy with a consequent rise in the elderly population, resulting in enhanced health and social costs. Cardiovascular diseases (CVD) rise with aging and are...
one of the foremost causes of fatality all over the world. Numerous risk factors have been characterized for CVD, but atherosclerosis, hypertension, insulin resistance and dyslipidemia are considered to be the most crucial factors associated with higher incidence of CVD [1–3].

The sirtuins (Silent information regulator of transcription or SIRT), a family of enzymes, have recently been identified as the pivotal regulators of lifespan and health [4]. Mammals possess seven sirtuins, encoded by SIRT1–SIRT7. As the exciting targets for CVD management, Sirtuins are expressed broadly in the nucleus and cytoplasm of multiple tissues and highly expressed in the heart tissue and the vascular endothelium [5]. SIRT1 is the best-known member of the sirtuin family which belongs to the sirtuin family of nicotinamide adenine dinucleotide (NAD+)–dependent protein deacetylases, whose activation appears beneficial for aging associated metabolic, inflammatory, and cardiac diseases, and to increase lifespan in model organisms [6].

SIRT1 has protective effects against CVD and through its deacetylase activity plays pivotal roles in regulating numerous biological functions including regulation of energy, detoxification of oxidative stress, promotion of angiogenesis, intracellular Ca²⁺ handling, promoting cell survival and longevity and induction of autophagy [7]. SIRT1 exerts its function through deacetylation of important substrates such as p53, Forkhead box class O (FOXO) transcription factors, peroxisome proliferator activated receptor (PPAR) co-activator 1a (PGC-1a), nuclear factor (NF)-κB and others, which are all closely linked to the age-related diseases including CVD [6]. Cardiac-specific upregulation of SIRT1 has extensively been shown to prevent atherogenesis, premature cardiac hypertrophy, apoptosis, cardiac fibrosis, cardiac dysfunction, and expression of cellular senescence markers, which increases with age [8–10]. However, SIRT1 inhibition has been associated with developmental abnormalities, including septal and valvular heart defects, vascular dysfunction, arterial thrombosis and alterations in fibrinolysis [11].

SIRT1 is mapped on chromosome 10 (10q21.3) and polymorphic variants of this gene have been studied in the susceptibility of several disorders, such as CVD [12], Type 2 diabetes (T2D) [13,14], glucose tolerance [15], overweight [16], lipid profiles and coronary artery [17], body fat and blood pressure [18], carotid plaque [19] and aging [20]. Promoter region variants may account for differential SIRT1 expression, rendering individuals susceptible to certain pathologies. Two SIRT1 rs3758391 T/C and rs369274325 G/T polymorphisms have been identified near the 5' end of the SIRT1 gene. Up until now, the rs3758391 T allele has been associated with T2D [14] as well as aging [20], however, no study has yet evaluated the impact of these two polymorphisms on the risk of CVD. Therefore, due to the involvement of SIRT1 in physiological processes underlying protection against CVD, the aim of this study was to analyze the SIRT1 promoter region polymorphisms rs3758391 T/C and rs369274325 G/T in relation to genetic susceptibility to CVD.

2. Subjects and methods

2.1. Patients and clinical data collection

In the present case–control study, a total of 250 CVD patients and 250 healthy subjects were recruited for the genotyping of SIRT1 gene polymorphisms. The CVD patients were recognized as the presence of identified myocardial infarction, coronary insufficiency (unstable angina with demonstrated ischemic electrocardiographic changes), death due to CHD, or atherothrombotic stroke. Blood samples were taken after 12 h overnight before cardiovascular procedures. Once registered, the participants filled questionnaire forms including race/ethnic status, demographics and history of cigarette smoking, hypertension and diabetes mellitus (DM) (Table 1). Hypertension was determined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg or therapy for hypertension. DM was diagnosed as a fasting blood glucose level >7 mmol/L or current use of hypoglycemic agents [19]. The study was approved by the institutional review board of cardiovascular institute, and the participating hospitals. The local Ethics Committee of the Zahedan University of Medical Sciences endorsed the project, and written informed permission was taken from all participants. The work is carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. DNA preparation and PCR-based allele genotyping

Blood samples were collected in EDTA-containing tubes and genomic DNA was isolated from peripheral blood cells by the salting-out method. TheSIRT1 rs369274325 G/T and rs3758391 T/C polymorphisms were genotyped by Tetra amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) as well as restriction fragment length polymorphism (RFLP) methods, respectively. The T-ARMS-PCR method employs two external primers, forward outer (FO) and reverse outer (RO) producing a control band and two allele-specific internal primers (RI and FI). This method simultaneously amplifies both alleles in one single-PCR tube. All primer sequences and fragments size are listed in Table 2.

PCR was performed by commercially available Prime Taq premix (Genetbio, South Korea) based on the manufacturer recommended protocol. Into a 0.2-mL PCR tube with a final volume of 20 μL, 1 ng of template DNA, 1 μL of each primer (10 μM), 10 μL Taq premix and 6 μL DNase-free water were added. The Cycling conditions for the SIRT1 rs369274325 G/T were a primary denaturation at 94 °C for 5 min followed by 30 cycles of 45 s at 94 °C, annealing temperature for 45 s at 59 °C and 45 s at 72 °C, with a final extension of 5 min at 72 °C. The PCR conditions for rs3758391 T/C polymorphism were 5 min at 94 °C followed by 30 cycles of 45 °C for 45 s, 56 °C for 45 s, 72 °C for 45 s followed by a final extension step for 5 min at 72 °C. PCR products were segregated using standard electrophoresis on a 2% agarose gel containing 0.5 μg/mL ethidium bromide and images were taken under UV light. The products size for SIRT1 rs369274325 G/T was 229 bp for the G allele and 152 bp for the T allele, whereas the product size for the two outer primers (control band) was 336 bp (Fig. 1). The amplicon size for the rs3758391 T/C was 241 bp, and the PCR product was digested with restriction enzyme HinII. After the RFLP enzyme digestion, two 146 bp and 95 bp (T allele) products were produced from the 241 bp amplicon (C allele) (Fig. 2).
2.3. Statistical analysis

Statistical analyses were executed using the SPSS program for Windows version 18.0 (SPSS, Chicago, IL, USA). Statistical analyses of continuous variables of demographic data were evaluated by Student’s t-test. Genotypes and allele frequencies were computed using the \( X^2 \) or Fisher probability test statistic. ORs and 95% CIs were calculated with the use of binary logistic regression analyses. Adjusted ORs were stratified by age, sex, ethnicity, history of CVD, hypertension, DM and current smoking. A two-sided \( P \)-value less than 0.05 was considered statistically significant.

3. Results

The present study demonstrated that the demographics characteristics of CVD patients and healthy controls were statistically different which included age, sex, ethnicity, history of CVD, hypertension, DM and smoking status (Table 1). The frequency distribution of the SIRT1 rs3758391 T/C and rs369274325 G/T genotypes and alleles was compared between 250 CVD cases and 250 controls.
As shown in Table 3, a significant difference was found between two groups regarding genotyping distribution of the SIRT1 at position rs3758391 T/C in either of additive and recessive models. In the additive model, the frequency of rs3758391 CC vs. TT genotype was increased in CVD patients than in the controls (19% vs. 6%), showing a statistically significant difference (OR = 3.06, 95% CI: 1.57–5.98, \( P = 0.001 \)).

Likewise, the recessive model indicated that the carriers of the CC genotype conferred a higher risk of CVD compared with those carrying the TT + CT genotype (OR = 3.72, 95% CI: 2.02–6.85, \( P = 0.001 \)). At the allelic level, although the C allele was more prevalent in the patients group than in the controls (45% vs. 39%), the difference did not reach the borderline of statistical significance (OR = 1.28, 95% CI: 0.99–1.64, \( P = 0.063 \))

On the other hand, the allelic and genotypic distributions of the SIRT1 polymorphism at position rs369274325 G/T were not significantly different between CVD patients and controls with the minor allele frequency (MAF) of 0 in both cases and controls (\( P = 1.00 \)).

Table 4 shows the analysis of demographic data of patients in relation to SIRT1 rs3758391 C/T genotypes. The analysis revealed that the SIRT1 CT + CC genotype was more frequent in male subjects than in women, although the difference was not statistically significant (\( P = 0.054 \)).

Additionally, no association among other risk factors of CVD and distribution of SIRT1 rs3758391 genotypes was found (\( P > 0.05 \)).

**4. Discussion**

Sirtuins are a unique class of proteins that link the acetylation status of proteins to a wide variety of diseases including CVD. SIRT1 exerts its cardioprotective activities through mediating...
SIRT1 plays an essential role in controlling blood pressure through regulating endothelial nitric oxide (NO) and endothelial nitric oxide synthase (eNOS). SIRT1 and eNOS co-localize and co-precipitate in endothelial cells (ECs), and SIRT1 deacetylates eNOS, inducing eNOS activity and mounting endothelial NO [8]. On the contrary, suppression of SIRT1 in the endothelium of arteries has been shown to reduce endothelial dependent vasodilation and bioavailable NO [22]. However, resveratrol (RSV), a SIRT1 activator, activates eNOS, improves endothelial function, restores vascular eNOS activity; hence prevents elevation of blood pressure in animal models of endothelial dysfunction [6,23]. Trans-resveratrol (trans-3,4,5-trihydroxystilbene, RSV) is a polyphenol compound which mimics the cardiac beneficial functions of SIRT1. RSV has advantageous effects against ischemia/hypoxia of cardiomyocytes and protects the heart from ischemic-reperfusion injury through the involvement of SIRT1 [24]. The other critical role of SIRT1 is reducing atherosclerosis by mediating several pathways. EC upregulation of SIRT1 increases eNOS-derived NO and has anti-inflammatory functions in ECs and macrophages, downregulating the expression of several proinflammatory cytokines by interfering with the NF-κB signaling pathway. Indeed, SIRT1 deacetylates ReA/p65 NF-κB proteins in macrophages and also inhibits expression of lectin-like oxidized low-density lipoprotein (ox-LDL) receptor (Lox-1), a scavenger receptor for ox-LDL, preventing macrophage foam cell formation, hence retarding atherosclerosis [8]. Furthermore, SIRT1 activates transcription of the liver X receptors (LXR) target gene encoding the ATP-binding cassette transporter A1 (ACTA1), which mediates HDL synthesis, reverses cholesterol transport and decreases the risk of atherosclerotic and cardiovascular events [6].

In this study we found a significant association of CVD patients with rs3758391 T/C polymorphism in SIRT1 gene. We found that the CC genotype of SIRT1 rs3758391 polymorphism was a risk factor for CVD in both additive and recessive models, suggesting that the carriers of the CC genotype were at 3- or 3.7-fold increased risk of CVD than subjects with the TT genotype, respectively. In accordance with our findings, the SIRT1 rs3758391 has been associated with the risk of a variety of diseases including T2D [14], systemic lupus erythematosus (SLE) morbidity [25] and aging [20]. Cruz et al. [14], observed a significant association of SIRT1 rs3758391 and T2D, suggesting this polymorphism as a risk factor for T2D in a Mexican population. Likewise, Zhang et al. [20] found the rs3758391/CC to be more common than rs3758391/CT and rs3758391/TT in the aging subjects with the OR of 3.042, which supports our findings in CVD.

The SIRT1 rs3758391 T/C polymorphism is located in the promoter region, within a putative p53 binding site. The rs3758391 C allele has been shown to result in a lower binding affinity for p53 compared to the T allele suggesting that this variant may be functional. In this context, TT and CT genotypes were shown to be associated with an increased SIRT1 expression in skeletal muscle of overweight individuals compared to the CC genotype [26]. In our study, the CC genotype (low SIRT1 producer) was more frequent in CVD patients than in controls, and considering the cardioprotective effects of SIRT1, this increased frequency may underlie the predisposition of patients to CVD.

Cardiac-specific expression of SIRT1 has also shown protection against oxidative stress in the heart tissue through hampering the myocytes cell death induced by Poly (ADP-ribose) polymerase-1 (PARP) activation. Intense activation of PARP by oxidative stress has been associated with a reduced SIRT1 deacetylation activity, and underlies the myocyte cell death contributing to heart failure. In contrast, SIRT1 activators such as RSV, induced Mn-SOD, and reduced oxidative stress participating in cardiomyocyte protection [8,27]. Thus, SIRT1 activators such as RSV could be considered as novel therapeutical tools for the treatment of chronic heart failure.

Another important cardioprotective role of SIRT1 is inducing cardiac cell survival and growth. Through decreasing DNA damage, apoptosis, and cell senescence, SIRT1 plays a critical role in cardiac development and myocardial cells growth to maintain cardiac function [8]. Conversely, Sirt1 knockout mice exhibited developmental abnormalities, including septal and valvular heart defects, emphasizing the SIRT1 role in pathological hearts [11].

Up till now, only a few human genetic studies of SIRT1 polymorphisms in CVD have been published, and our finding is the first report suggesting the association of SIRT1 rs3758391 T/C polymorphism with susceptibility to CVD. In conclusion, our study suggests that the SIRT1 rs3758391 CC genotype may confer an increased risk for the CVD in both additive and recessive models in this Iranian population. Additional researches on larger populations are warranted to validate our results.

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References


