The effect of the *SIRT1* 2827 A > G polymorphism, resveratrol, exercise, age and occupation in Turkish population with cardiovascular disease

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Abstract

Objective: Cardiovascular disease (CVD) is the leading cause of death in Europe. One of the candidate molecule affecting epigenetic mechanisms of CVD is the *SIRT1*, a subclass of sirtuins, is located on the long arm of chromosome 10 (10q21.3). Particularly, the relation between 2827 A>G polymorphism of the *SIRT1* positioned on exon 2, leading to conversion of histidine to arginine, and the formation of CVD is not known yet. One of the activator of *SIRT1*, resveratrol, is also known as a cardioprotective molecule. On the other hand, the parameters including exercise, occupation and age affect CVD. The aim of the present study was to investigate the effect of the rs144124002 (2827 A>G) single nucleotide polymorphisms (SNP) of *SIRT1* and exercise-occupation-age parameters on CVD.

Methods: SNP of *SIRT1* were analyzed using DNA isolation, the polymerase chain reaction (PCR) and restriction fragment length polymorphism. To do so, large cohorts of CVD patients (n=293) and healthy controls (n=117) who directed Cardiology Department of Bezmialem Vakıf University, Bezmialem Vakıf University Hospital were used.

Results: In this study, when we assessed CVD and control groups about 2827 A>G polymorphism, all individuals were determined as homozygous genotype. We found a positive effect between the modifications of resveratrol, exercise, age and occupation and CVD (OR=0.17; CI 95%, 0.1-0.2; $p \leq 0.001$).

Conclusion: This is the first study demonstrating the correlation between the *SIRT1* rs144124002 polymorphism and CVD in Turkish population. *(Anatolian J Cardiol 2015; 15: 103-6)*

Key words: *SIRT1*, resveratrol, epigenetic, polymorphism, cardiovascular disease

Introduction

Cardiovascular disease (CVD), the leading cause of death in Europe (1), occurs by interactions of environmental and genetic factors. The prevalence of CVD is 21.7% in Turkish population (with 29 age of onset) (2). In the previous candidate gene, linkage analysis and genome-wide association studies, more than a dozen of genes and genetic loci related with CVD were identified (3, 4). However, elucidating the underlying molecular mechanism of genetic causes for CVD demands more investigations.

The silent mating type information regulation-2 (Sir2) family proteins are the product of a 7-member gene called the *SIRT* and function as the nicotinamide adenine dinucleotide dependent protein deacetylases (Class III). In mammals, this protein is called as sirtuin (5). The *SIRT1* is located on the long arm of chromosome 10 (10q21.3) and consists of 9 exons and 8 introns (6). The 2827 A>G polymorphism is positioned on exon 2 leading to a histidine-to-arginine conversion (7).

Sirtuins play a fundamental role as a key regulator in a wide variety of cellular processes like cell defense and survival, aging, transcription, gene silencing, DNA repair, cell cycle progression, apoptosis, inflammation, and stress resistance (8, 9). *SIRT1* is activated by exercise, wine and plant compounds such as resveratrol (10-12). Resveratrol, a natural phenolic compound produced by the grapes and a few other plant species, has anti-inflammatory and anticancer effects in various organs, as well as having a cardioprotective activity (13, 14). However, to determine the safety and optimal dose of resveratrol, further research and more clinical studies are necessary.

This study is pronounced as an abstract on 10. Medical Genetics Congress.
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The aim of the present study was to investigate the association between rs144124002 (2827 A>G; 69647253: CAT→CGT; His→Arg) SNP of *SIRT1* with CVD and the protective role of resveratrol in Turkish population. The PCR and RFLP were used to determine the *SIRT1* polymorphism. The relationships between CVD and the lifestyles of participants like their nutritional behavior and exercise habits were also evaluated.

Methods

Study design and participants

The subjects were selected from patients who had a CVD, diagnosed at the Department of Cardiology in Bezmialem Vakıf University Hospital, and undergone an angiography. In the present study, there were 293 cases with CVD and 117 healthy controls. The mean ages for the CVD patients and the control group were 59.0±10.6 years (range was 33-86 years) and 49.2±6.6 years (range was 32-76 years), respectively. The age range for group was 33-86 years. The control group comprised the volunteers with no CVD in themselves or their relatives. The data of participants who had nutritional habits, occupation and exercise habits were collected as a questionnaire form by their physicians. According to these data, study population was divided into different categories as active or passive occupation groups and exercise habits. We also divided patients with CVD into another two groups consuming red grape resveratrol more than 250 gr (for resveratrol) per week and not consuming.

The current study and all experimental procedures were approved by the Local Ethics Committee of the Bezmialem Vakıf University, İstanbul, Turkey. A written informed consent was obtained from each participant.

Sample preparation

The venous blood samples were taken from subjects and from these samples; 5 mL of blood was transferred to tubes containing EDTA. The samples were transported on ice to the Research Center. After DNA isolation, the blood samples including EDTA were stored +4°C.

Genotype assessment

DNA isolation of the blood samples collected from both groups was performed by a precipitation method using a saturated commercial DNA isolation Kit (Invitrogen, K182002). The *SIRT1* gene was analyzed by PCR-based RFLP method. This region multiplied with PCR as 150 base pairs (bp) by using primers designed using a primer design program, i.e., these are forward 5'GCCTTGACTGACTTGGTTTCTT 3' and reverse 5'CATACCTATCCGTGGCCTTG 3'. 100 ng genomic DNA in 25 µL reaction mixture containing 2.5 µL 10 X PCR buffer (Thermo Scientific, # B33), 200 µM dNTP (Thermo Scientific, # R0191), 10 pM each of primers, and 5 Unit (U) of Taq DNA polymerase (Thermoscientific, # EP0701) was used. Optimal amplification of heat cycles in the PCR reaction were determined as 3 minutes at 98°C for a frontal denaturation, 45 seconds at 95°C for denaturation, 30 seconds at 65°C for an adhesion, 30 seconds at 72°C for synthesis, and 5 minutes for a total of 30 cycles and final synthesis at 72°C. After amplification, the PCR products were stored at 4°C. As a next step, 150 bp PCR product was digested with *NlaIII (Hin1II)* (Neb) enzyme in a 25 µL reaction solution including 2.5 µL of 10 X buffer, and 5 U of *NlaIII (Hin1II)* at 37°C overnight. The cut zone of the *NlaIII (Hin1II)* enzyme is CATG. For these polymorphisms, the region which was multiplied 150 bp with PCR was divided into two fragments of 105 and 45 bp.

Statistical analysis

The results were presented as a percentage. The statistical analyses were performed using SPSS for Windows version 13.0 (SPSS, Chicago, IL, USA) program. Continuous variables are expressed as mean±SD or median (interquartile range) when appropriate. To compare categorical variables, Pearson chi-square test was used. A 'p' value less than or equal to 0.05 was considered as statistically significant.

Results

The relation between SIRT1 polymorphism and CVD

Initially, the allele frequencies regarding *SIRT1* 2827 A>G polymorphism in CVD patients and control subjects were compared. The genotype frequencies of AA in the CVD patients and controls were 100%. In the present study, the adjusted p value between the patients and controls for the 2827 A>G polymorphism was statistically insignificant, showing no relationship between the risk of CVD and the *SIRT1* 2827 A>G polymorphism.

The relation among SIRT1 polymorphism, gender, age and occupation

Table 1 demonstrates the relationship between CVD patients and controls as to gender, age, exercise, and usage of resveratrol as grape. In the ≤55 age group, we found the frequencies of CVD in patients and controls 50% and 50%, respectively, and for the >55 age group, 85.5% and 14.5%, respectively (Table 1). The statistical difference between the CVD patients and controls for age group is highly significant (OR=0.17; CI 95%, 0.1-0.2; p≤0.001).

The frequencies of CVD patients and controls in the active occupation group (characterized by requiring a standing posture: being in physical motion) were 81.5% and 18.5%, respectively, and for the passive occupation group (characterized by requiring a sitting posture: a sedentary occupation), 68% and 32%, respectively (Table 1). A statistically significant difference was found between the CVD patients and controls for job group (OR=2.07; CI 95%, 1.13-3.795; p=0.016).

The effect of exercising more than 1 hour a week resulted in a significant decline in risk for CVD patients (OR=2.5; CI 95%, 1.4-4.7; p=0.001). The impact of taking resveratrol more than 250 gr per week as grape was statistically significant between the CVD patients and controls for plant compounds (OR=2,2; CI 95%, 1.25-4.09; p=0.006) (Table 1).

Table 1. Frequencies of age, occupation, exercise, and resveratrol groups for CVD-controls

		CVD-control		
		CVD group	Control group	*P*
Age, years group	≤55 n (%)	106 (50)	106 (50)	0.001*
	>55 n (%)	171 (85.5)	29 (14.5)	
Occupation group	Active n (%)	75 (81.5)	17 (18.5)	0.016*
	Passive n (%)	138 (68)	65 (32)	
Exercise group	More than once a week	42 (64.6)	23 (35.4)	0.001*
	None	223 (82.6)	47 (17.4)	
Resveratrol group	More than once a week	47 (67.1)	23 (32.9)	0.006*
	None	218 (82.3)	47 (17.7)	

*Pearson chi-square test

Discussion

In this study, we have investigated the association of the polymorphisms of the *SIRT1* with increased risk of CVD and the protective role of resveratrol on CVD.

When we analyzed the effect of 2827 A>G at the *SIRT1*, no statistically significant relation was found between the aforementioned polymorphism and CVD or other parameters. The prevalence of *SIRT1* 2827 A>G polymorphism reported in our study is similar from the results revealed from ESP Cohort population studies whose data is derived from population cohorts participating in the National Heart, Lung and Blood Institute (NHLBI) Exome Sequencing Project studied North America. This study declared that the prevalence of the AA, AG and GG genotypes are 0.998, 0.002 and 0.0 respectively (HapMap database, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=144124002). There is not any investigation for *SIRT1* 2827 A>G polymorphism. However, only a few human genetic association studies on *SIRT1* (except for our mutation) have been published so far. *SIRT1* genetic variation is related to obesity (15). Furthermore, these studies reported that *SIRT1* rs7895833 and rs7069102 polymorphisms are associated with CVD (16). Nomiyama et al. (17) declared that SIRT1 activates liver X receptor (LXR). LXR operates as cholesterol sensor to protect the organism from cholesterol overload and reduces cholesterol loading in macrophages, and so protecting against atherosclerosis. Therefore, carriers of rs7069102 and rs2273773 could have reduced activities of SIRT1 and LXR (18). The other study is Shimoyoma's study which is about sirtuin 1 gene polymorphisms are associated with cholesterol metabolism and coronary artery calcification in Japanese hemodialysis patients. According to this study, the different allele frequencies in rs7895833 and rs7069102 between hemodialysis patients and controls could have an impact on survival (19).

As mentioned in a previous study, a fiber of the diet, grape contains resveratrol behaves as various cardioprotective antioxidants and the consumption of the resveratrol has been linked to reduced cardiovascular diseases (20). Moreover, many studies have assessed that the positive relationship between sedentary life and CVD (21). The previous studies also demonstrated that the cardioprotective effect of resveratrol occurs by its role in glucose homeostasis and lipid metabolism in mice (22). Therefore, further clinical investigations are needed for resveratrol efficacy in CVD. In the current study, we also found a statistically significant effect of resveratrol, age, occupation, exercise groups between CVD patients and control groups.

Study limitations

The resveratrol or the congeners of resveratrol are appealing enhancing attention as possible agents for CVD chemoprevention. This study was not a study to determine the optimal dose of resveratrol. Therefore, further clinical investigations are needed to determine the dose of resveratrol for resveratrol efficacy in CVD.

Conclusion

In conclusion, the *SIRT1* and its protein product have an important role in the risk of CVD. From the viewpoint of the CVD and control groups, *SIRT1* 2827 A>G polymorphism is statistically insignificant differences. Furthermore, resveratrol and exercise have positive cardioprotective effects. Definitive correlation would require further studies.

Conflict of interest: None declared.

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