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Association of Endonuclease G Gene Variants with Cardiovascular Disease Risk Factors

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Abstract

Background: Cardiovascular disease (CVD) is a leading cause of death, supporting the need for the identification of novel biomarkers as risk stratification factors. Endonuclease G (ENDOG) has recently been suggested to be a novel determinant of cardiac hypertrophy and mitochondrial function, and plays an important role in apoptosis processes involved in cardiac myocyte death. The aim of current study was to explore the association of two genetic variants in ENDOG gene (*ENDOG*) with CVD risk factors in an Iranian population.

Methods: Subjects included 663 patients with CVD and 282 healthy individuals recruited as part of the Mashhad Stroke and Heart Atherosclerotic Disorders Cohort Study. The *ENDOG* S12L (rs 2293969) and L142M (rs 61397314) variants were genotyped. Anthropometric and biochemical factors were measured in all the subjects followed by univariate and multivariate analyses to determine the association of these genetic markers with CVD and biochemical parameters.

Results: *ENDOG* polymorphisms were found at a significantly higher prevalence in individuals who had histories of smoking and breaking point in L142M. In contrast, other risk factors for cardiovascular disease, including lipid profile and blood pressure, showed no or very weak relationship with the *ENDOG* polymorphisms.

Conclusions: Our findings indicated an association between an *ENDOG* genetic variant and smoking history as a cardiovascular risk factor. Further studies in the prospective setting are warranted to investigate the value of this marker.

Keywords: Cardiac Vascular Diseases, Endonuclease G, Polymorphism.

Introduction

Cardiovascular disease is the leading cause of death globally (1). Myocardial infarction, ischemic heart disease, hyperlipidemia, reperfusion injury, and heart failure are the most prominent causes of cardiovascular disease (2). Apoptosis, or programmed cell death, plays an important role in these factors that lead to cardiovascular disease (3, 4). Several studies have shown that apoptosis is important following a myocardial infarction (5). One of the most important apoptotic pathways is caspase-independent (6), which utilizes intermembrane proteins that are released from the mitochondrial inter-membranous interval into the cytosol (7). Several factors are involved in this process, including endonuclease G (ENDOG) and apoptosis-inducing factor (AIF) (8). These factors mediate apoptosis and cause DNA fragmentation without activation of the caspase cascade. A

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growing body of data shows that the expression of ENDOG-like 1 gene (*ENDOGL1*) is ubiquitous with relatively abundant expression in the brain and pancreatic islet beta cells (9, 10). *ENDOG* is a candidate disease-susceptibility gene for type 2 diabetes in the Japanese population (11). Furthermore, Takeshita et al. investigated the genotype distribution of the *ENDOG* polymorphisms in 13 various Asian, African, and Caucasian populations, and found that of the four *ENDOG* SNPs, S12L may be a crucial polymorphic marker (12). The goal of the present study was to investigate the association of two *ENDOG* genetic polymorphisms with coronary artery disease (CAD) in a large population from the Mashhad Stroke and Heart Atherosclerotic Disorders Cohort Study.

Materials and methods

Study participants

Six hundred and sixty-three patients (416 men and 272 women) with cardiovascular disease and 282 healthy controls (83 men and 199 women) were recruited from Mashhad University of Medical Science (MUMS) during March 2014 to March 2015. Patients with signs or symptoms of cardiac disease including chest pain, ECG changes, unstable angina, and angina of effort admitted to the emergency ward of Ghaem hospital and subsequently diagnosed with CVD were enrolled in this study. Individuals with known chronic cardiovascular disease, more than one heart attack, history of angioplasty, or long-term cardiac drug administration were excluded from the study. Patients with chronic diseases including cancers and autoimmune diseases were also excluded. Informed consent was obtained from all participants using the protocol approved by the Medical Ethics Committee of the Mashhad University of Medical Sciences.

Anthropometric Measurements

Anthropometric parameters, including height, weight, and waist and hip circumferences (HC) were measured for all cases.

Lipid Profile of Population

Lipid profile levels, including total cholesterol (TC), fasting blood glucose, triglyceride, low-density lipoprotein (LDL), and high-density lipoprotein cholesterol (HDLC) were measured using standard procedures.

DNA Isolation and Genotyping

Genomic DNA was extracted from blood using kits (Favor Gene Corp, Taiwan) according to the manufacturer's protocol. Genotyping of *ENDOG* S12L (rs 2293969) and L142M (rs 61397314) was performed using RFLP-PCR as described previously. The primer sequences are shown in Table 1 (18). PCR amplification was performed in 20 µl reaction mixtures with 5 ng of DNA. Each reaction tube contained 2 µL of 10X reaction buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl), 1-2 µL of 25 mM MgCl2 solution, 0.5 µL dNTPs mix (10 mM of each), 1 µL of each forward and reverse primer, 0.25 µL Taq (5 units/µL), 5 µL of template DNA, and 10 µL of sterilized distilled water (15).

Table 1. PCR primers used to detect *ENDOG* SNPs.

SNP	Primer	Sequence (5′-3′)
S12L (rs2293969)	2293969-F	ACCGCACCCAGCCCCGCTCC
	2293969-R	AGCCCTGTGCCCTCGTTGGAT
L142M(rs61397314)	61397314-F	AAGAGCCGCGAGTCGTACGTGCT
	61397314-R	TTCTGGCTCCAGCGGTGGTT

Amplification was performed with a protocol consisting of initial denaturation at 96 ºC for 10 min followed by 30 cycles with denaturation at 96 ºC for 30 s, annealing at 65 ºC for 30 s, and extension at 74 ºC for 30 s, followed by a final extension at 74 ºC for 10 min. To confirm amplification, we electrophoresed the product in a 2% agarose gel with 100 bp DNA ladder (Cat. No. PR901644). Ten µL of the PCR product was digested with 1-2 µL of Taq 1 (New England Biolab, UK) restriction enzyme, 2 µL of 10X Taq 1 buffer containing 10 mM Tris-HCl, pH 7.5 at 25 °C, 300 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 mg/mL BSA, and 50% glycerol, and incubated

for one hr at 65 ºC. Digested products (20 µl) were electrophoresed on an 8% polyacrylamide gel with 10 bp DNA ladder, and the products were visualized by silver staining. To verify our genotyping result, products were sequenced by the dideoxy chain-termination method.

Statistical analysis

Statistics were analyzed using SPSS 20 (SPSS Inc. USA). Descriptive statistics including mean, frequency, and standard deviation (SD) were determined for all variables and expressed as means ± SDs for normally-distributed variables or as medians and IQRs for not normally-distributed variables. The student's t-test was used for normally-distributed variables. The Mann–Whitney U test was used for continuous variables if they were not normally distributed. Chi-square or Fisher exact tests were used for categorical variables. Logistic regression analysis was used to calculate association between polymorphisms and CVD. All the analyses were two-sided and statistical significance was set at $P < 0.05$.

Results

Demographic data including age, gender, hypertension, and cardiovascular history, and patient clinical characteristics are shown in Table 2. Interval of anthropometry variables of participants is also shown in Table 2. The median male weight was 72.73 kg with a confidence interval of 71.61-73.84 kg, and the median female weight was 66.45 kg with a confidence interval of 65.24-67.66 kg. The average heights of men and women were 1.66 and 1.55 m, respectively. Table 3 shows the genotyping result of both SNPs studied in this research. We used binary logistic regression to determine how the independent variable can predict the presence of the restriction site in the participants. In this method, before analyzing the independent variable, the background effect of age, gender, or other independent variables that were not requisite were removed and data normalized. Binary logistic regression of haplotypes 1 and 2 by independent variables in the patient group are shown in Table 4. The restriction site was predicted in 65.5% of patients. Of all the variables, smoking history in candidates was most significantly associated with *ENDOG* SNPs, as patients with smoking history 42.7 for S12L (rs2293969) and 65.6 for L142M (rs61397314) showed more sensitivity than non-smokers to the restriction enzyme digestion. In addition, a significant association was found between diabetes as a risk factor for cardiovascular disease and presence of the Taq 1 restriction enzyme site.

Table 2. Subjects 'clinical characteristics

Variables		N (%)
Gender	Male	500 (53%)
	Female	445 (47%)
Age	29-50	181 (29.5)
	50-79	764 (80.8)
Angiography	Positive	663 (70%)
	Negative	282 (30%)
Smoking	Yes	193 (22%)
	No	748 (80%)
Physical activity	Inactive	651 (70%)
	Moderate	282 (30%)
	Active	12 (1.3%)
Hypertension history	Yes	447 (47%)
	No	498 (53%)
Hyperlipidemia history	Yes	361 (38%)
	No	584 (62%)
Cardiovascular history	Yes	433 (46%)
	No	512 (54%)
Diabetes history	Yes	355 (38%)
	No	590 (62%)

Table 3. Means and 95% confidence intervals of anthropometry variables

Variables	Gender	Mean	confidence interval
Weight (Kg)	Male	72.73	71.61-73.84
	Female	66.45	65.24-67.66
Height (M)	Male	1.66	1.65-1.67
	Female	1.55	1.54-1.56
BMI (Kg/m²)	Male	26.48	26.10-26.85
	Female	27.70	27.14-28.26
Waist circumference (cm)	Male	93.64	92.57-94.71
	Female	95.29	94.06-96.52
Hip circumference (cm)	Male	100	99.25-100.75
	Female	101.51	100.66-102.36

Table 4. Genetic information of population

SNP	AA	AG	GG	A%	G%
S12L (rs2293969)	434	354	157	0.641	0.353
L142M (rs61397314)	435	389	121	0.666	0.334

Discussion

In the cardiovascular system, apoptosis has been found in ischemic and idiopathic dilated cardiomyopathies, myocardial cell death, long-QT syndrome, and arrhythmogenic right ventricular dysplasia (3). The exact role of apoptosis in the pathophysiology of ischemic heart disease is unclear, although several studies have shown an association between apoptosis and cardiovascular risk factors, including hypertension and hypercholesterolemia (13, 14). DNA fragmentation factor beta polypeptide (DFFB), ENDOG, and flap endonuclease-1 (FEN-1) are known to be responsible for DNA fragmentation (12).

ENDOG in mice is a mitochondrion-specific nuclease that translocates to the nucleus during apoptosis. Once released from mitochondria, ENDOG cleaves chromatin DNA into nucleosome fragments independently of caspases (10, 15). Therefore, ENDOG represents a caspase-independent apoptotic pathway initiated by the mitochondria (16). Investigations have implicated the role of this restriction enzyme in progression and development of cancer, aging, and heart and neurodegenerative diseases (17).

Cardiovascular disease and its risk factors have a distinct relation with ENDOG. In 2011 an *ENDOG* mutation was identified that results in loss of function and may be associated with increasing left ventricular mass and impaired cardiac function. Inhibition of *ENDOG* in cultured cardiomyocytes resulted in increased cell size and hypertrophic biomarkers in the absence of pro-hypertrophic stimulation (18, 19).

Furthermore, Takeshita et al. investigated the genotype distributions of both rs2293969 and rs61397314 in worldwide populations including three ethnic groups. They concluded that the *ENDOG* genes showed relatively low genetic diversity, suggesting that these endonucleases have been well conserved at the protein level during human evolution. Moreover, several studies have indicated that *ENDOG* expression was significantly increased in response to carbamylated low-density lipoprotein (cLDL) exposure. Moreover, developers of a region-wide case-control test concluded that *ENDOGL1* is a candidate gene in susceptibility to type 2 diabetes (20).

In the current study, we examined *ENDOG* SNPs in 945 subjects to determine whether ENDOG is an apoptotic factor in coronary artery disease. Our data indicated that smoking history in both case and control groups induced a caspase-independent apoptosis pathway. Furthermore, studies showed that smoking induced anti-apoptosis factors that can inhibit apoptosis in both caspase-dependent and -independent pathways (21). Because the case subjects in this study had coronary artery disease and the control subjects did not, we cannot authoritatively conclude that smoking leads to coronary artery disease by inhibiting apoptosis factors. Therefore, our data suggested that a larger population with participants with coronary artery disease who smoke

and a control group of healthy non-smokers is needed for a final conclusion.

Our findings indicated an association between the *ENDOG* S12L (rs 2293969) and L142M (rs 61397314) SNPs and cardiovascular risk factors. Cigarette smoking and diabetes type 2 had the highest correlations of the risk factors analyzed while hypertension and hypercholesterolemia had the lowest. Further studies with a larger population will be needed to evaluate ENDOG function as a prognostic factor in cardiovascular diseases.

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