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Importance of *NPC1* Gene 644 A→G Mutation in Coronary Artery Disease

Sibel Demir Ozturk[1], Atac Celik[2], Ayse Feyda Nursal[3], Akin Tekcan[4], Aydin Rustemoglu[5], Nevin Karakus[6] and Serbulent Yigit[7]

[1,5,6,7]Gaziosmanpasa University, Faculty of Medicine, Department of Medical Biology, 60100, Tokat, Turkey
[2]Gaziosmanpasa University, Faculty of Medicine, Department of Cardiology, 60100, Tokat, Turkey
[3]Hitit University, Faculty of Medicine, Department of Medical Genetic, 19100, Corum, Turkey
[4]Ahi Evran University, Faculty of Medicine, Department of Medical Biology, 40100, Kirsehir, Turkey

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ABSTRACT Coronary artery disease (CAD) is the most prominent cause of mortality worldwide. The basis of CAD pathogenesis is the occlusion of coroner vessels progressively due to atherosclerotic plaques. *NPC1* gene plays a critical role in the atherosclerosis progression. This study aimed to examine whether 644 A→G polymorphism of *NPC1* is associated with the risk of coronary artery disease in Turkish patients. In this case-control study, 200 persons were studied (100 patients and 100 controls). The 644 A→G polymorphism of *NPC1* gene is analyzed using polymerase chain reaction and restriction fragment length polymorphism methods. There was a significant relationship between the distribution of coronary artery disease and control group in terms of allele and genotype frequency (p= 0.0002) (p=0.003), respectively. According to the researchers' results, 644 A→G polymorphism in *NPC1* gene can be one of the predisposition factor to coronary artery disease in Turkish population.

INTRODUCTION

Coronary artery disease (CAD) is a serious and chronic disorder that is characterized by the narrowing and remodeling of the coronary artery. CAD is the important reason for morbidity and mortality in worldwide. Atherosclerosis has the leading role in the pathogenesis of CAD (Zhang et al. 2014). Atherosclerosis is described as a disease characterized by the accumulation of low-density lipoprotein (LDL)-derived lipids in the arterial intima. And, it is a chronic inflammatory case that is progressed due many risk factors prominently dyslipidemia (Riccioni et al. 2012).

Niemann-Pick type C (NPC) disease is a rare, autosomal recessive, multi-systemic, fatal and neurodegenerative disorder that is characterized by lysosomal accumulation of unesterified cholesterol, resulting from mutations in one of two genes, either *NPC1* or *NPC2* (Millard et al. 2005; Vanier 2015). NPC occurs by mutations in *NPC1* gene (95%) or NPC2 gene (5%). *NPC1* gene encodes 1278 amino acid protein that is one of the membrane-bound proteins. This protein has roles in the transport of cholesterol from late lysosomes to other compartments in the cell. *NPC1* mutations lead to aberrant lipid transportation from endocytic compartments and this cause's lysosomal storage of lipids; particularly cholesterol and glycosphingolipids (Liscum 2000). Dysfunction of lipoprotein metabolism contributes to atherosclerotic disease via alterations in arterial lipid accumulation and atherosclerotic plaque formation (Sakellarios et al. 2013). *NPC1* gene has been shown to play a critical role in the atherosclerotic progression. The aim of this study was to examine the association between the *NPC1* gene polymorphism and CAD in a Turkish population.

Address for correspondence:
Akin Tekcan
Professor
Ahi Evran University, Faculty of Medicine
Department of Medical Biology, 40100,
Kirsehir, Turkey
Telephone: +90505 571 9646
E-mail: akintekcan@hotmail.com

METHODOLOGY

Study Design

In this study, 100 patients diagnosed with CAD (77 males, 23 females, study group) and 100 healthy volunteers (64 males, 36 females, healthy control group) who were recruited to Tokat Gaziosmanpasa University, Department of Cardiology were included in the study. This study was conducted in Gaziosmanpasa University, Department of Medical Biology and Genetics laboratories. Both the study group and control group were recruited from the Turkish population. Subjects included in the study were over the age of 18. Informed written consent was obtained from all patients and subjects before enrollment to the study, according to the ethical guidelines of the 2008 Declaration of Helsinki and the investigation was approved by the ethical, investigation and biosecurity committee of Gaziosmanpaºa University, Faculty of Medicine.

Genotype Determination

NPC1 644 A→G polymorphism was genotyped by the restriction fragment length polymorphism (RFLP) technique. In this study, the researchers have used Fermentas Genomic DNA Purification Kit. DNA isolation was performed according to manufacturer's protocol. The PCR-RFLP technique that was used by Ma et al. (2010) was modified for *NPC1* gene +644 A→G polymorphism.[6] Forward primer; 52 GGGTTGCCTTG-GTATGTG-32 , Reverse primer; 52 - ATCGTC-CAGGGAGCAG-32 . Genomic DNA was amplified in a 25-ìl final reaction. Polymerase chain reaction (PCR) cycles were 95°C (5 minutes) for 1 cycle, followed by 94°C (20 seconds), 60°C (30 seconds), and 72°C (35 seconds) for 30 cycles. A final cycle of 72°C for 5 minutes completed the reaction. Amplified PCR product was digested in a 30-ìl final reaction volume using 2 ìl of Reaction Buffer and 1 ìl of Nco I restriction enzyme at 37°C 30 minutes. Controls of known genotype were included for every set of digestions carried out. The digested products were resolved on 2% agarose gel stained with ethidium bromide and visualized using ultraviolet transilluminator (UV).

Statistical Analysis

All statistical analyses were performed using computer SPSS Statistical Program Version 13.0 and OpenEpi info 2.2 software package program. Continuous data were given as mean±SD (standard deviation) and (min-max). x^2 test was used for significance of differences in the allele frequency and genotype distribution between the two study groups. Hardy-Weinberg equilibrium test was performed for both study groups. Odds ratio (OR) and 95 (%) confidence intervals (CIs) were calculated. *P* value $p<0.05$ was considered statistically significant.

RESULTS

Allele frequencies were measured in 100 patients and 100 controls for this biallelic polymorphism. Hardy-Weinberg equilibrium was maintained in both patients and controls. The demographic characteristics of the CAD patients and controls are shown in Table 1. Hypertension is an important characteristic of CAD patients ($p<0.001$, Table 1) compared with heathy controls. Also, the other characteristics of CAD patients such as gender, family story, smoking and diabetes mellitus are not different from healthy controls ($p> 0.05$, Table 1) in this study. There was no difference between the mean age of patients and that of controls. The mean age was 39-78 in patients and 35-80 in control group, respectively. There were 23 percent females and 77 percent males in the patient group, in the control group it was 36 percent and 64 percent, respectively. Males constituted the majority of cases in both patient and control groups respectively. The researchers did not find any association with smoking in CAD and control groups. There was a statistically significant difference between CAD and control groups regarding hypertension values ($p=0.001$). A statistically

Table 1: Baseline clinical and demographic characteristics of the patients with CAD and healthy controls

Characteristics	*CAD (%)*	*Control group (%)*	*p*
Mean age	62.12	59.30	
Women	23%	36%	0.044
Male	77%	64%	
Family story	36%	31%	0.275
Smoking	15%	28%	0.019
Hypertension	73%	49%	**0.001**
Diabetes Mellitus	28%	52%	0.314

The results that are statistically significant are typed in bold.

significant difference between CAD and control groups with respect to LDL levels were shown (p=0.001). The serum lipid levels belong to CAD and control group can be seen in Table 2. There was no association regarding clinical features in CAD group (Table 3). There was a significant difference related to allele and genotype frequency in A/G polymorphism in CAD and patient group. The frequency of the *NPC1* 644 A→G polymorphism can be seen in Table 4.

Table 2: Lipid profiles of CAD and control groups

	CAD (%)	*Control group (%)*	*p*
LDL (mg/dl)	113.94	132.67	**0.001**
HDL (mg/dl)	44.69	45.12	0.801

The results that are statistically significant are typed in bold. **LDL:** Low-density lipoprotein, **HDL:** High-density lipoprotein

Table 3: Clinical characteristics of CAD patients group

Clinical characteristics	*p*
Sex	0.586
Age	0.395
Family story	0.534
Smoking	0.417
Hypertension	0.051
Diabetes Mellitus	0.889
HDL	0.819
LDL	0.290
TG	0.454

LDL: Low-density lipoprotein, **HDL:** High-density lipoprotein, **TG:** Triglyceride

DISCUSSION

In this study, the researchers have aimed to examine difference of *NPC1* 644 A'!G polymorphism between CAD and control groups in Turkish population. CAD is an important social problem that can lead to fatalities all around the world and also in Turkey. Atherosclerosis is a multifactorial and systemic disease that starts in the beginning of the life and increases over the life course. NPC disease is an inherited lipid storage disorder. NPC1 gene mutation can be seen in 95 percent of the NPC patients. *NPC1* gene mutation leads to the accumulation of lipids in lysosomes and in late endosomes and disrupts the intracellular lipid transportation. It is shown that *NPC1* heterozygote mice have abnormal metabolic features such as hyperinsulinemia, glucose intolerance and increase in susceptibility to weight gain (Jelinek et al. 2011). Robiou-du-Pont et al. (2013) have stated that *NPC1* gene polymorphism was related to insulin resistance according to their study performed with patients with Diabetes Mellitus Type-2 in French society (Robiou-du-Pont et al. 2013). The results of genome-wide study conducted in European population have suggested that *NPC1* gene polymorphism was the risk factor for obesity in juvenile/adult morbid obese individuals compared to people with normal weight (Meyre et al. 2009). Similarly, Sandholt et al. (2011) have indicated that *NPC1* rs1805081 had roles in predisposition to obesity and increased body mass index (BMI) although it was not related to Diabetes Mellitus Type-2 and lipid levels (Sandholt et al. 2011).

According to GWAS study, Xi et al. (2013) and Wu et al. (2010) have not shown any relationship between *NPC1* rs1805081 and central obesity/body mass index (BMI) and generalized obesity in Chinese obese children (Wu et al. 2010; Xi et al. 2013). Mejia-Benitez et al. (2013) have revealed that *NPC1* rs1805081 had a prominent relationship with rapid glucose increase and decrease

Table 4: Genotype and alleles frequencies of *NPC1* gene +644 AG in CAD and control groups

NPC1 +644 AG	*CAD (n=100)(%)*	*Controls (n=100)(%)*	*p*	*OR (CI 95%)*
Genotypes				
AA	35 (35)	52 (52)		
AG	33 (33)	35 (35)	**0.003**	
GG	32 (32)	13 (13)		
AA+AG : GG	68 (68): 32 (32)	87 (87): 13 (13)	**0.001**	3.13 (1.54-6.61)
AA : AG+GG	35 (35): 65 (65)	52 (52): 48 (48)	**0.015**	2.01 (1.14-3.56)
Alleles				
A	103 (51.5)	139 (69.5)	**0.0002**	2.14 (1.42-3.24)
G	97 (48.5)	61 (30.5)		

The results that are statistically significant are typed in bold. **OR:** Odds ratio

in Mexican children (Mejía-Benítez et al. 2013). These studies conducted with different populations have supported the idea that *NPC1* gene mutations or polymorphisms lead to distinct alterations in hepatic lipid homeostasis that induced weight gain and insulin resistance. It is known that the cholesterol mechanism is disrupted in Alzheimer's disease. Depending on this information, the epistasis was studied between *NPC1/ABCA1* genes that belonged to cholesterol trafficking-related genes and Alzheimer's disease. In this study, it has been observed that the risk for Alzheimer's disease was found to be high in individuals who carried both *ABCA1* (-477) TT and *NPC1* exon 6 GG, *NPC1* intron 20 AA, *NPC1* intron 22 AA or *NPC1* intron 24 GG genotype (Rodríguez-Rodríguez et al. 2010).

The relationship between the *NPC1* gene 644 A/G polymorphism and CAD disease is not much studied. Ma et al. (2010) have studied the association between CAD and *NPC1* gene variations as well as the relationship between these variations regarding smoking or nonsmoking. The relationship between *NPC1* gene polymorphism and coronary heart disease has been investigated by Ma et al. (2010). First of all, they examined the *NPC1* 644 A/G gene polymorphism in Chinese coronary heart disease patients (Ma et al. 2010). They detected the frequencies of G allele homozygote and heterozygote genotypes were significantly lower in patients with coronary heart disease than in control subjects. In this study, they have also compared the smokers and non-smokers in terms of coronary heart disease. The frequencies of both homozygote and heterozygote G alleles in patients who smoke have been found lower both in recessive (GG vs. AA+AG) and additive (GG vs. AG vs. AA) model when compared to non-smokers. It has been also shown that *NPC1* variant G allele in 644 A/G polymorphism has protective roles in CAD in smokers in Chinese population (Ma et al. 2010). Also, *NPC1* gene 644A>G, 1926C>G, 2572A>G and 3797G>A polymorphisms are investigated in terms of overweight and gestational diabetes and, it was found that 2572A>G polymorphism was closely associated with overweight and gestational diabetes (Garver et al. 2015).

The researchers study is the first study to examine the relationship between the *NPC1* gene and CAD in Turkish population. There was a significant difference regarding the allele and genotype frequency between CAD and control groups. G allele and GG genotype were detected more in CAD group compared to control group. This study indicated that the variant allele G at position 644 in *NPC1* gene is associated with increased risk of CAD.

CONCLUSION

In this study, the researchers have evaluated the relationship between CAD disease and *NPC1* gene polymorphism which has role in lipid metabolism. Early intervention was recommended on the high risk individuals who carrying more risk alleles in lipid metabolism-related genes. Additional analyses with larger populations are required to confirm these findings in different study populations.

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