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Original Article

Effects of common variations of NOS3 and CAV1 genes on hypercholesterolemic profile in coronary heart disease

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ABSTRACT

Caveolin-1 (CAV-1) plays a crucial role in endothelial-nitric oxide synthase (eNOS) enzymatic activity. Therefore, CAV-1 and eNOS interactions have a significant impact on endothelial dysfunction, cholesterol levels, and atherosclerosis. We investigated the critical variations in *NOS3* and *CAV1* genes in this case–control study to determine the relations between the coronary heart disease (CHD) risk factors. The NOS3-rs1799983, CAV-1 rs3840634, and rs3807990 variations were analyzed in 76 CHD patients and 91 controls using the polymerase chain reaction. Mean serum Total-cholesterol levels were significantly higher in CHD patients with the CAV-1 rs3807990-T allele than in patients with CC genotype (p=0.017). There was a statistically significant correlation between the rs3807990-T allele and hypercholesterolemia in the CHD group (p=0.008). The multivariate analysis confirmed that the CAV-1 rs3807990-T allele (p=0.011) is a risk factor for hypercholesterolemia. Moreover, the serum HDL-Cholesterol level was detected to be higher in patients carrying both CAV1-rs3807990-T and NOS3-rs1799983-T alleles than those with the CAV-1 rs3807990-CC/NOS3-rs1799983-GG genotype subgroup (p=0.013). These results suggested that the genetic variations of CAV-1 rs3807990 and NOS3-rs1799983 may contribute to the increased hypercholesterolemia risk and thus on the development of CHD.

Keywords: Cav1, NOS3, gene, hypercholesterolemia, lipid, CHD

INTRODUCTION

Coronary heart disease (CHD) was among the leading causes of mortality in worldwide. Endothelial dysfunction plays a central role in atherosclerosis pathogenesis that leads to CHD (Foy and Grant 1997; Hadi and Suwaidi 2007). Main causes of endothelial dysfunction are impaired anticoagulant and antiplatelet mechanisms, increased production of cellular adhesion molecules and increased vascular tone due to reduced bioavailability of endothelial-derived vasodilatory nitric oxide (NO) (Lahera et al. 2007). In the mammalian blood vessels, most of the NO production is mediated by calcium-calmodulin controlled endothelial nitric oxide synthase isoenzyme (eNOS). Caveolin-1 (Cav-1) encoded by the Cav-1 gene (Grilo et al. 2006), is a permanent regulator of the eNOS enzyme activity (Ju et al. 1997; Blair et al. 1999). Cav-1 converts the inactivated form of eNOS by binding directly to the oxygenase domain and Ca^{+2}/CaM activate the eNOS in the endothelial cells (Ju et al. 1997).

Cav-1 is found in macrophages, and endothelial and vascular smooth muscle cells (VSMC) take part in the atherosclerosis process (Couet et al. 1997). A remarkable decrease was observed in the extent of atherosclerotic lesions in a study on $ApoE^{-/-}$ $Cav\text{-}1^{-/-}$ double knockout mice (Puglielli et al. 1995). In contrast, it was reported that the reduced Cav-1 expression in VSMCs of atheroma,

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which suggested that it might have an anti-atherogenic effect in VSMCs (Williams and Lisanti 2004). Cav-1 functions as a scaffold protein for the organization and activation of proteins such as Src-like kinase, G proteins and eNOS in caveolae membrane (Marsden et al. 1993; Chen et al. 1996). It was shown that Cav-1 may cause atherosclerosis by inactivating eNOS in endothelial cells in several experiments on mice. Endothelial nitric oxide synthase enzyme encoded by the *NOS3* gene has "N-terminal oxygenase" and "C-terminal reductase" domains and the Ca^{+2}/ calmodulin (CaM) binding region is located between these two domains (Venema et al. 1996; Yoshimura et al. 1998).

Cav-1 and caveolae also have roles in maintaining cholesterol homeostasis. While Cav-1 interacts with the sterol carrier protein-2 (SCP-2) involved in the delivery of newly produced cholesterol from the smooth endoplasmic reticulum to the cell membrane (Chen et al. 1996; Lusis 2000), caveolae are effective in taking the released free cholesterol to plasma by the HDL and cholesterol esters from plasma (Frank and Lisanti 2004). Both the amino-terminal and carboxy-terminal cytoplasmic regions of Cav-1 and the oxygenase domain of eNOS are involved in this interaction. Recent studies have reported that the deficiency of Cav-1 in endothelial cells (ECs) impairs LDL transendothelial migration. Therefore, the pro-atherogenic effects of Cav-1 in endothelial cells are mainly attributed to its effects on LDL transcytosis. Unlike ECs, Cav-1 deficiency in macrophages causes the foam cells formation by stimulating the accumulation of cholesterol esters. This observation suggests that it has an antiatherogenic effect (Hassan et al. 2004). Therefore, it was suggested that the Cav-1 had differently influenced the development of atherosclerotic vascular disease depending on cell type and metabolic pathway.

Studies regarding the association between the Cav-1 gene variants with cardiovascular diseases and lipid metabolism are scarce (Chen et al. 2005; Schwencke et al. 2005; Carey et al. 2012). Chen et al. 2005, reported that rs3807989 at the Cav-1/Cav-2 locus was associated with significant risk of coronary artery disease (CAD) and myocardial infarction (MI) by increasing Cav-1 expression. They also suggested that "A" allele of the *CAV1* rs3807989 gene is correlated with a decreased LDL cholesterol level.

There is transversion mutation (G>T) at nucleotide 1917 in exon 7 of the *NOS3* gene, causing the glutamic acid to aspartic acid substitution at codon 298 (Glu298Asp, rs1799983) (Huang et al. 1995; Conde et al. 2006). The Glu298Asp mutation has been shown to be related to atherosclerotic coronary events (Huang et al. 1996; Freedman et al. 1999; Lefer et al. 1999; Huang 2000). Although the interactions of Cav-1 and eNOS activity have been shown in previous studies (Chen et al. 1996; Venema et al. 1996), there are limited studies concerning the association of the genetic alterations in these genes. Joshi et al. have shown that the Cav-1 - NOS3 complex is dissociated to notably much more in the Glu/Glu wild type ECs than in the Asp variants. They suggested that NOS3 Glu298Asp variation changed caveolar localization and damaged endothelial response to shear in human endothelium (Joshi et al. 2007).

In a recent study, the rs3840634 (2 bp deletion) and rs3807990 C>T variants of Cav-1 together with *NOS3* rs1799983 were shown to be related to colorectal cancer susceptibility (Conde et al. 2006). Shyu et al. (2017) demonstrated the minor alleles of genotypic polymorphisms of the *NOS3* rs1799983 G>T (Glu-298Asp), Cav-1 rs3807987 G>A and rs7804372 T>A are related to the increased stroke risk of large artery among Han Chinese. Due to the relationship between Cav-1 and eNOS, we suggest that functional genetic variations of these proteins could be an important risk factor for cardiovascular events. Thus, the aim of this study was to examine the association between the *NOS3*-rs1799983, Cav-1 rs3840634, and rs3807990 gene variations and the risk of CHD with respect to both of individual and combined effects of lipid profiles and the other atherosclerotic risk factors in the Turkish population. Regarding Cav-1, this is the first study investigating the effects of the Cav-1 rs3807990 SNP on risk parameters in CHD patients.

MATERIALS AND METHODS

Subjects

This study was a case-control investigation. Control group consisted of 91 healthy individuals with no family history and any signs of diabetes mellitus, renal failure, hypertension, or dyslipidemia. The patient group consisted of 76 patients diagnosed with coronary heart disease followed by the Department of Cardiology, İstanbul University Faculty of Medicine between the period from 2013 to 2014. All the patients were receiving statin for lipid-lowering effects based on conventional therapy.

Criteria for angiography were 50% stenosis at least in one main coronary artery caused by atherosclerosis, and a vascular case, described as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. All subjects gave the answers for the full histories with special emphasis on coronary risk factors like family history, diabetes mellitus, hyperlipidemia, hypertension, and smoking. Percentages of patients with hypertension, type 2 diabetes and left ventricular hypertrophy were 43.4%, 43.4%, and 39.6%, respectively.

This study was arranged in accordance with the "World Medical Association Declaration of Helsinki" and written consents were obtained from all participants. This study was authorized by the Ethics Committees of the Faculty of Medicine, İstanbul University.

Genotyping

Genomic DNA was isolated from the peripheral blood samples collected in EDTA tubes by using a DNA isolation kit (Roche Diagnostics GmbH, Mannheim, Germany). *CAV1* rs3840634 (AC deletion) genotyping was carried out by LightCycler real-time PCR (Roche, Germany) via commercial LightSNiP assays from TIB-MolBiol (Germany), in accordance with the company's procedures. Melting curve analysis of the PCR products qualified the genotypes of the rs3840634 as homozygote major allele (TT), heterozygote (CT) and homozygote minor allele (CC). Tm values were 56.98 and 63.77 for the T and C alleles, respectively.

Cav-1 rs3807990 (C>T) and *NOS3* rs1799983 (G>T) polymorphisms were determined by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method using the MspI and BanII restriction enzymes, respectively. MspI

Table 1. Characteristics of the study groups

	Control n=91	CHD n=76	p
Age (year)	59.10±9.85	59.49±12.38	0.822
Sex (Women/Men) (n)	38/53	24/52	0.175
SBP (mmHg)	123.99±12.27	135.95±32.38	0.003
DBP (mmHg)	73.91±8.54	83.99±17.18	0.0041
TC (mmol/L)	4.72± 0.96	5.28± 1.40	0.003
TG (mmol/L)	1.45± 0.66	1.77± 1.80	0.123
HDL-C(mmol/L)	1.22± 0.34	1.03± 0.21	0.001
LDL-C (mmol/L)	2.86± 0.87	3.23± 0.81	0.006
VLDL-C (mmol/L)	0.71± 0.39	0.72± 0.32	0.819
BMI (kg/m2)	25.83± 2.33	25.58± 4.20	0.669
Smoking (%)	21.8%	59.5%	0.001
Alcohol consumption (%)	4.7%	16.7%	0.030
CHD Family history (%)	21.7%	33.9%	0.285

Values were derived by using independent-samples. The results are shown as mean ± SD. Bold values of p indicates statistical significance. TC, total cholesterol; TG, triglyceride; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; VLDL-C, VLDL-cholesterol; TC/HDL-C, total cholesterol/ HDL-cholesterol; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; n, number of individuals.

Table 2. Genotype distributions of the *CAV1* and *NOS3* variations in the study groups

CAV1 and *NOS3* variations		Study Groups	
CAV1 rs3807990		Controls (n=91)	CHD patients (n=73)
Genotypes	CC	49 (53.8%)	46 (63%)
	TT	8 (8.8%)	5 (6.8%)
	CT	34 (37.4%)	22 (30.1%)
	HWE	p=0.552 (p>0.05)	p=0.300 (p>0.05)
Alleles	C	132 (72.5%)	114 (78.1%)
	T	50 (27.5%)	32 (21.9%)
CAV1 rs3840634			
Genotypes	TT	91 (100%)	73 (100%)
	CC	0 (0%)	0 (0%)
	CT	0 (0%)	0 (0%)
	HWE	Uncountable	Uncountable
NOS3 rs1799983			
Genotypes	GG	44 (48.35%)	33 (44%)
	TT	7 (7,69%)	8 (10.7%)
	GT	40 (43.96%)	32 (45.3%)
	HWE	p=0.726 (p>0.05)	p=0.826 (p>0.05)
Alleles	G	122 (67.03%)	98 (67.12%)
	T	54 (29.67%)	48 (32.88%)

n: number of samples, HWE: Hardy-Weinberg Disequilibrium.

digestion resulted in fragments of 122 and 121 bp for the C allele of *CAV1* rs3807990 (C>T). PCR product of the *CAV1* rs3807990 (C>T) (243 bp) was not digested with MspI in the presence of the T allele. The *NOS3* gene rs1799983 (G>T) Glu298Asp missense mutation causes a BanII recognition site, that digests the PCR product to fragments of 198 and 122 bp in the presence of the G allele and a single fragment of 320 bp for the T allele. The digestion products were separated by 3% agarose gel electrophoresis. Homozygous normal GG genotype had two bands (198 and 122 bp) while the homozygous mutant TT genotype had a 320 bp band after visualized under UV. Heterozygous GT genotype has three bands like 320 bp, 198 bp, and 122 bp.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) software package version 20.0, (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Values of $p<0.05$ were accepted statistically significant. The odds ratio and 95% confidence interval were worked out to examine relative risks among the study groups. Comparing the quantitative data among groups were carried out by applying Student's t-Test and ANOVA test, for more than two variables. Compatibility with the Hardy-Weinberg equilibrium (HWE) of the comparisons of genotype and allele were analyzed by chi-square test. Gene counting methods were used for calculating the allele frequencies.

The Binary logistic regression analysis was used to assess the effects of the *CAV1* rs3807990 SNP on hypercholesterolemia in patients with CHD (Table 6). In the regression analysis, the *CAV1* rs3807990 SNP T allele, sex, and Type 2 Diabetes mellitus and smoking were used as independent variables. The multivariate regression model including hypercholesterolemia status as the dependent variable was used.

RESULTS

Clinical Investigation

Demographic and biochemical characteristics of the study groups are given in Table 1. As expected, the CHD group had a higher prevalence of conventional cardiovascular diseases risk factors such as systolic blood pressure (SBP) (p=0.003), diastolic blood pressure (DBP) (p<0.001), Total-cholesterol (Total-C) (p=0.003), LDL-cholesterol (LDL-C) (p=0.006), smoking (p<0.001) and alcohol consumption (p=0.03) and lower HDL-cholesterol (HDL-C) levels (p<0.001). There were no statistical differences in age, sex, body mass index (BMI), triglyceride and

VLDL-cholesterol (VLDL-C) levels and family history of CHD between the patient and control groups (p>0.05).

Distributions and Metabolic Effects *CAV1* rs3807990 and *NOS3* rs1799983 Genotypes

As shown in Table 2, the Cav-1 rs3807990 and *NOS3* rs1799983 gene variations were concordant with HWE in both groups (p>0.05). We also analyzed the Cav-1 gene rs3840634 AC deletion. However, the rare C allele of the Cav-1 rs3840634 was not found in either group and all individuals were carrying the normal TT genotype. Therefore, compliance with HWE was not evaluated for the Cav-1 rs3840634 variants.

When the effects of the Cav-1 rs3807990 genotypes on serum lipid profile, BMI, and blood pressures were investigated in study groups, BMI levels were higher in individuals with the CT genotypes than with the TT genotype in the control group (p=0.046) and DBP value was lower in Cav-1 rs3807990 normal C allele carriers than in non-carriers (TT genotype) (C allele: 73.42±8.65, TT genotype: 79.67±4.32, p=0.014) (Table 3). In the patient group, CT genotype carriers had higher serum Total-C levels than CC genotype carriers (p=0.008, one way Anova test) (Table 3). In addition, it was found that Total-C were higher in patients with the T allele than in those with the CC genotype (T allele: 5.78±1.88, CC genotype: 4.98±0.90, p=0.017, Student's t test) (Table 3). Therefore, increased levels of Total-C observed in the patients carrying the CT genotype can be attributed to the T allele.

When the effects of the *NOS3* rs1799983 genotypes on serum lipid profile, BMI, and blood pressures in the study groups were examined, BMI value was found to be higher in controls with the TT genotype as compared to the GG and GT genotype carriers (p=0.003) and G allele carriers (p=0.002). However, in the patient group, there were no statistically important effects of the *NOS3* rs1799983 genotypes on clinical or biochemical parameters (p>0.05) (Table 4).

The combined effects of Cav-1 rs3807990 and *NOS3* rs1799983 genotypes were also evaluated on serum lipids, BMI, and blood pressures which are given in Table 5. In CHD patients with the Cav-1 rs3807990 rare T allele and the *NOS3* rs1799983 rare T allele higher HDL-C levels were observed than patients with the common genotypes of Cav-1 rs3807990 (CC) / *NOS3* rs1799983 (GG) (1.12±0.12 vs. 1.01± 0.22, p=0.013). There were no significant differences found among the rare T alleles of Cav-1 rs3807990 / *NOS3* rs1799983 haplotype and the Cav-1 rs3807990 CC/*NOS3* rs1799983 GG haplotype in terms of serum lipid profile in the controls (p>0.05).

The association between the Cav-1 rs3807990 SNP and hypercholesterolemia (serum Total-C above 5.18 mmol/L) in the patients with CHD is shown in Figure 1. A significant association was observed between the rs3807990 rare T allele and hypercholesterolemia (chi-square= 6.951, p=0.008). However, we didn't observe any effect of this SNP on hypercholesterolemia in the controls (p=0.161), as it was observed in CHD patients.

Table 3. Metabolic parameters for the CAV-1 rs3807990 genotypes among study groups

Groups	rs3807990				
	CC	CT	TT	C allele (CC+CT)	T allele (TT+CT)
Control	n=49	n=34	n=8	n=83	n=42
Age	59.78±10.36	58.71±9.60	56.63±8.02	59.34±10.01	58.31±9.27
Glucose	94.64±10.10	96.94±12.19	105.00±7.55	95.48±10.85	98.04±11.87
Total-C (mmol/L)	4.66±1.06	4.84±0.88	4.59±0.61	4.74±0.99	4.80±0.84
TG (mmol/L)	1.38±0.55	1.57±0.80	1.41±0.62	1.46±0.66	1.54±0.77
HDL-C (mmol/L)	1.20±0.28	1.19±0.36	1.42±0.52	1.20±0.31	1.23±0.40
LDL -C(mmol/L)	2.79±0.91	3.03±0.84	2.47±0.56	2.89±0.88	2.93±0.82
VLDL-C(mmol/L)	0.69±0.42	0.74±0.38	0.70±0.34	0.71±0.40	0.73±0.37
BMI (kg/m2)	25.94±1.96	26.05±2.51*	24.23±3.17	25.99±2.19	25.71±2.71
SBP (mmHg)	123.29±12.84	125.27±12.11	122.67±9.93	124.09±12.50	124.83±11.67
DBP (mmHg)	73.02±7.50	74.00±10.19	79.67±4.32	73.42±8.65¥	74.97±9.65
CHD Patients	n=46	n=22	n=5	n=68	n=27
Age	61.46±10.75	58.50±14.30	54.60±15.82	60.50±11.99	57.78±14.35
Glucose	179.35±121.15	174.72±130.63	223.33±184.81	177.83±123.13	181.67±134.99
Total-C (mmol/L)	4.98±0.90	5.92±2.04&	5.13±0.63	5.29±1.43	5.78±1.89∑
TG (mmol/L)	1.55±0.67	2.23±3.13	1.89±1.10	1.77±1.9	2.16±2.85
HDL-C (mmol/L)	1.03±0.20	1.06±0.16	1.12±0.21	1.04±0.19	1.07±0.17
LDL -C(mmol/L)	3.07±0.82	3.47±0.69#	3.33±0.81	3.20±0.79	3.44±0.70 Ω
VLDL-C(mmol/L)	0.72±0.31	0.72±0.30	0.72±0.49	0.72±0.30	0.72±0.34
BMI (kg/m^2)	25.75±4.07	24.96±3.43	22.91±3.70	25.47±3.85	24.52±3.51
SBP (mmHg)	133.64±25.78	145.0±41.94	116.0±37.82	137.42±32.30	139.62±42.10
DBP (mmHg)	82.84±17.13	88.41±17.42	74.0±15.17	84.69±17.29	85.74±17.68

The results are shown as mean ± SD. *, p=0.046; ¥, p=0.014; &, p=0.008; ∑, p=0.017; #, p=0.054; Ω, p=0.053.

Table 4. Metabolic parameters for the NOS3 rs1799983 genotypes among study groups

GROUPS	rs1799983				
	GG	GT	TT	GG/GT	TT/GT
CONTROL	n=44	n=40	n=7	n=84	n=47
Age	58.49±9.96	59.66±9.66	56.67±12.48	59.04±9.76	59.18±10.02
Glucose	97.06±11.96	94.73±12.62	99.00±7.53	95.80±12.20	95.48±11.87
Total-C	4.65±1.15	4.80±0.64	4.95±1.00	4.72±0.94	4.82±0.69
TG	1.35±0.49	1.48±0.78	1.36±0.81	1.41±0.64	1.46±0.76
HDL-C	1.29±0.35	1.20±0.38	1.15±0.31	1.25±0.36	1.19±0.37
LDL -C	2.84±0.99	2.94±0.62	2.80±0.95	2.89±0.83	2.91±0.67
VLDL -C	0.62±0.25	0.74±0.42	0.85±0.82	0.67±0.34	0.76±0.49
BMI	25.44±1.99	25.35±2.70	28.59±2.19*	25.40±2.34¥	25.86±2.89
SBP	122.72±11.80	125.13±13.14	113.60±10.71	120.03±12.50	123.48±13.33
DBP	72.40±7.65	77.03±9.37	72.60±9.15	74.92±8.86	76.40±9.34
CHD PATIENTS	n=33	n=32	n=8	n=65	n=40
Age	61.09±11.84	59.24±13.63	56.38±6.93	60.15±12.72	58.69±12.61
Glucose	194.48±134.92	191.19±140.49	135.50±71.01	192.86±136.36	180.75±131.22
Total-C	5.29±1.64	5.24±1.27	5.35±1.07	5.27±1.45	5.27±1.23
TG	1.65±0.81	1.94±2.54	1.50±0.67	1.80±1.90	1.85±2.31
HDL-C	1.01±0.23	1.05±0.19	1.12±0.17	1.02±0.21	1.06±0.18
LDL -C	3.26±0.95	3.20±0.72	3.15±0.59	3.23±0.84	3.19±0.69
VLDL -C	0.74±0.36	0.68±0.27	0.75±0.34	0.72±0.32	0.70±0.28
BMI	26.16±4.48	24.98±4.00	26.46±4.03	25.53±4.23	25.32±4.00
SBP	142.34±31.90	134.09±33.66	121.25±26.42	138.15±32.82	131.59±32.49
DBP	86,72±16,29	83,18±16,85	78,13±22,35	84,92±16,55	82,20±17,86

The results are shown as mean ± SD. *, p=0,003; ¥. p=0.002.

Table 5. Combined effects of CAV-1 rs3807990 and NOS3 rs1799983 on metabolic parameters

GROUPS	CAV-1 rs3807990 and NOS3 rs1799983 Haplotypes		
	eNOS T allele/CAV-1 T allele	eNOS GG/CAV-1 CC	P value
CONTROL	n=22	n=69	
Glucose (mg/dL)	99.36±13.37	95.05±10.07	0.250
Total-C (mmol/L)	4.77±0.66	4.75±1.04	0.943
TG (mmol/L)	1.62±0.91	1.39±0.54	0.288
HDL-C (mmol/L)	1.19±0.44	1.23±0.30	0.586
LDL -C(mmol/L)	2.91±0.62	2.89±0.94	0.920
VLDL -C(mmol/L)	0.79±0.43	0.68±0.39	0.293
BMI (kg/m2)	25.27±3.14	25.87±2.01	0.432
SBP (mmHg)	126.26±14.77	123.27±11.81	0.380
DBP (mmHg)	76.53±10.94	73.65±7.29	0.206
CHD PATIENTS	n=16	n=56	
Glucose (mg/dL)	194.75±141.84	186.02±132.13	0.842
Total-C (mmol/L)	5.81±1.60	5.12±1.34	0.084
TG (mmol/L)	2.44±3.66	1.57±0.71	0.357
HDL-C (mmol/L)	1.12±0.12	1.01±0.22	0.013
LDL -C(mmol/L)	3.53±0.64	3.13±0.84	0.089
VLDL -C(mmol/L)	0.70±0.32	0.71±0.32	0.885
BMI (kg/m2)	25.25±3.62	25.75±4.43	0.693
SBP (mmHg)	129.38±39.41	139.75±30.38	0.313
DBP (mmHg)	81.56±18.05	85.27±17.01	0.451

The results are shown as mean ± SD. Bold values of p indicates statistical significance.

Table 6. Evaluation of risk factors associated with hypercholesterolemia by Binary logistic regression analysis in CHD patients

Variables	p value	B	SE	Exp (B)	OR (95% CI) for Exp (B)
Sex	0.353	0.582	0.627	1.790	0.524-6.114
Type 2 Diabetes mellitus	0.480	-0.448	0.553	0.639	0.184-2.217
Smoking	0.850	0.133	0.700	1.142	0.290-4.504
CAV1 rs3807990 T Allele	0.011	-1.412	0.726	0.244	0.082-0.721

SE: Standard Error; OR: odds ratio; CI: Confidence Interval; (level of significance: p<0.05).

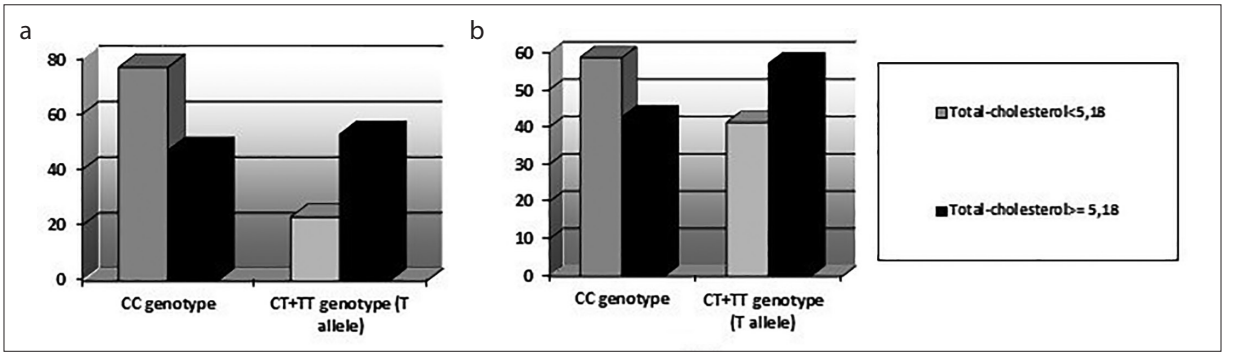

Figure 1. a, b. The association between the CAV1 rs3807990 SNP and hypercholesterolemia in (a) the patient and (b) the control group. *, chi-square= 6.951, p=0.008.

The effects of the risk parameters that we observed on the development of hypercholesterolemia in the CHD group were further evaluated by Binary logistic regression analysis (Table 6). The Cav-1 rs3807990 T allele, sex, diabetes mellitus, and smoking took part in the categorical variables in the risk analysis for hypercholesterolemia. As a result, it was confirmed that the Cav-1 rs3807990 variation was a risk of hypercholesterolemia in the patient group using logistic regression analysis (p=0.011).

DISCUSSION

Endothelial dysfunction is associated with cardiovascular risk factors like dyslipidemia, arterial hypertension, hyperglycemia, and diabetes mellitus (Foy and Grant 2007; Hadi and Suwaidi 2007; Lahera et al. 2007). Reduction of nitric oxide (NO) bioavailability underlies on the basis of the endothelial dysfunction (Drab et al. 2001). NO, synthesized from L-arginine by eNOS enzyme in the endothelial cell membrane, is an important factor blocking the atherosclerosis pathogenesis (Blair et al. 1999; Foy and Grant 2007). Several studies examined the effects of the eNOS gene variations on endothelial dysfunction and atherosclerosis pathogenesis in eNOS gene-deficient mice. Absence of the eNOS gene was shown to lead to lack of EDRF (Endothelial-derived releasing factor) activity, hypertension (Razani et al. 2001), leukocyte-endothelial adhesion (Hingorani et al. 1999), increment of platelet aggregation (Casas et al. 2004), vascular smooth muscle cell proliferation (Razani et al. 2001; Fedele et al. 2013), inclination to thrombosis, stroke (Colombo et al. 2002) and atherosclerosis (Shyu et al. 2017).

The eNOS enzyme activity is transcriptionally regulated by interaction with the presence of the substrate, calcium, calmodulin, enzyme cofactors such as FAD, FMN, NADPH, BH4 and with proteins such as Hsp90 and caveolins in various steps (Atochin et al. 2007). Direct interaction of eNOS and caveolin-1 inhibits the eNOS activity. It was shown in studies on mice lacking the Cav-1 gene that the eNOS activity was increased due to the lack of inhibition that should have been created by the interaction between Cav-1 and eNOS (Granath et al. 2001; Karvonen et al. 2002).

Much is known about the association of the *NOS3* rs1799983 (Glu298Asp) genotypes with the risk of atherosclerotic coronary events in humans, but the results are controversial. Many studies have suggested a relationship between this gene variation and cardiovascular risk (Huang et al. 1995; Huang et al. 1996; Lefer et al. 1999). In a meta-analysis including 26 studies and a total of 23028 samples, Casas et al. (2004) suggested that the risk of ischemic heart disease might be high in individuals with *NOS3* Asp298 and intron-4a homozygous genotypes. However, some studies yielded conflicting results (Huang 2000). Also, in our study, the *NOS3* genotype distribution was similar between the study groups (p>0.05). These different findings might be due to ethnic differences between populations and interactions between the G894T polymorphism and other polymorphisms. Additionally, these findings caused by other factors determining the activity of the eNOS enzyme may show a difference in various populations. When we examined the effects of *NOS3* rs1799983 variant on biochemical parameters such as serum lipid profile and blood pressure, we also found no significance between individuals with different genotypes in both groups. In the control group, BMI was observed higher in TT genotype carriers than in GT and GG genotype carriers. As a result of this study, we determined that the *NOS3* rs1799983 polymorphism individually could not lead to CHD or its risk factors.

There are only a few studies in the literature that have examined the *NOS3* and Cav-1 gene variations in cardiovascular diseases (Grilo et al. 2006; Joshi et al. 2007; Shyu et al. 2017). It was reported that the Cav-1/NOS3 complex is dissociated to a notably greater extent in the common Glu/Glu ECs than in the Asp variants (Joshi et al. 2007). They suggested that the *NOS3* Glu298Asp variation altered caveolar localization and impaired response to shear in human endothelium. Shyu et al. suggested that the minor alleles of the *NOS3* rs1799983 G>T, *CAV1* rs3807987 G>A and rs7804372 T>A are associated with the increased risk of large artery atherosclerotic stroke among Han Chinese (Shyu et al. 2017). Moreover, Grilo et al. (2006) showed an association between the Cav-1 rs3807990 mutant T allele and high systolic blood pressure, while they did not find any association with serum lipids and lipoprotein levels in their study. They also suggested that there was no gene-gene interaction between the *NOS3* and Cav-1 genes with regard to metabolic syndrome in hypertensive patients. However, Razani et al. (2001) have determined that Cav-1 - / - mice had higher plasma triglyceride levels compared to mice with normal genotype. Frank et al. (2006) investigated the relationship between Cav-1 and cellular cholesterol homeostasis and found that the Cav-1 molecule had a minimal effect on HDL and apolipoprotein A-mediated cholesterol efflux. They also found that the Cav-1 molecule had a crucial effect on the cellular cholesterol homeostasis regulation (Hassan et al. 2004). Smart et al. (1996) similarly suggested that the Cav-1 molecule may be an intracellular cholesterol raft. Formation of Caves and the expression of the Cav-1 protein depend on cholesterol. Caveolin proteins also take a hand in the intracellular cholesterol balance (Chen et al. 1996; Lusis 2000). Cav-1 protein has been shown to interact with SCP-2, which is responsible for the newly synthesized cholesterol delivery from the endoplasmic reticulum to the cell membrane (Chen et al. 1996). Also, Caves function in the process of taking up excess free cholesterol released to plasma into HDLs, the reverse transport of cholesterol, and also the removal of cholesterol esters from plasma (Frank et al. 2004). Our findings confirm that caveolin-1 may have an effect on serum lipid/lipoprotein levels. In our study, BMI was higher in controls with the Cav-1 rs3807990 CT genotype than the TT genotype (p= 0.046) and the normal C allele was observed to be correlated with low diastolic blood pressure (p=0.014).

Serum total-C levels have been shown to be higher in patients with both the CT genotype (p=0.008) and the T allele (TT genotype + CT genotype) (p=0.017) compared to the CC genotype. Therefore, our results indicate that the Cav-1 rs3807990 rare T allele may contribute to the high total–C (p=0.017) levels in patients with CHD.

We also determined that the *CAV1* rs3807990 rare T allele is associated with hypercholesterolemia (serum total-C>5.18 mmol/L) (p=0.008). In addition, logistic regression analysis confirmed the Cav-1 rs3807990 T allele was a significant risk factor in the development of hypercholesterolemia in CHD subjects. In this context, our study findings also support the association between serum cholesterol levels and the Cav-1 gene variations. Moreover, we investigated the combined effects of the NOS3 and Cav-1 gene polymorphisms in the present study. In CHD patients who had both the Cav-1 rs3807990 rare T allele and the *NOS3* rs1799983 rare T allele, the serum HDL-C level was found to be higher in the than the Cav-1 rs3807990 common CC/ *NOS3* rs1799983 common GG genotype carriers (1.12±0.12 vs. 1.01± 0.22, p=0.013). However, this association needs to be verified in larger study groups. We believe that our findings will be more meaningful and more concrete in a study group on a wider scale, and will guide the future work in this context.

The limitation of our study was the relatively small study groups (n=177). Secondly, some effects of Cav-1 polymorphisms on serum lipid levels may be masked because the individuals in the patient group received statin therapy in this study, Therefore, we suggest that the effects of Cav-1 gene variations will be more definitive in future studies in which we will investigate the effect of Cav-1 gene polymorphisms on serum lipid levels in patients with CHD receiving with and without statin therapy.

As a conclusion, a better understanding of endothelial dysfunction in the pathogenesis of atherosclerosis is essential for the development of new treatment modalities. Therefore, we investigated the critical variations in *NOS3* and Cav-1 genes in this study in order to determine the relations between the CHD risk factors and NO production in the endothelial function process. Our study indicated that the Cav-1 rs3807990 T allele may be one of the risk factors for the development of hypercholesterolemia and coronary heart disease.

Ethics Committee Approval: This study protocol was approved by the Ethics Committees of the Istanbul Faculty of Medicine, Istanbul University (approval number:2011/1276-643, date: 27th July, 2013).

Informed Consent: All participants received medical approval from their personal physicians and gave written, informed consent prior to giving their blood sample. This study protocol was arranged according to the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects".

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