ISSN 0015-5497, e-ISSN 1734-9168

Folia Biologica (Kraków), vol. 65 (2017), No 4
https://doi.org/10.3409/fb65_4.213

Effects of Common INSIG-2 and SCAP Gene Variants on Metabolic Parameters in Coronary Artery Disease

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Accepted December 08, 2017 *Published online December 29, 2017* *Published December 29, 2017*

COBAN D., ERONAT A.P., CEVIZ A.B., COSKUNPINAR E., BUGRA Z., YILMAZ-AYDOGAN H., OZTURK O. 2017. Effects of common INSIG-2 and SCAP gene variants on metabolic parameters in Coronary Artery Disease. Folia Biologica (Kraków) **65**: 213-221.

The function of sterol regulator element binding proteins (SREBPs), important transcription factors in cholesterol synthesis, is associated to SREBP cleavage activating protein (SCAP), insulin induced gene product-1 (INSIG-1) and INSIG-2. In this study, the possible contribution of INSIG-2 rs9308762 and SCAP rs4858868 SNPs to CAD risk was investigated in 73 CAD patients and 58 healthy controls. The systolic and diastolic blood pressure levels of patients were higher than in the control group (P<0.001), while HDL-C levels were lower (P=0.015). In the CAD group, the observed frequency of the SCAP GA genotype was higher (P<0.05), while INSIG-2 rs9308762 genotypes and alleles were not different between study groups. The INSIG2 rare T allele was found to be associated with BMI compared to the CC genotype (P=0.017). Logistic regression analysis confirmed that the INSIG2 T-allele is associated with high BMI levels in controls, while low serum HDL-C in CAD patients. Based on our findings, it can be deduced that these INSIG2 and SCAP variants contribute to CAD risk.

Key words: SCAP, INSIG-2, gene, cholesterol, CAD.

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The relationship between high plasma total- and low-density lipoprotein cholesterol with atherosclerotic cardiovascular diseases is well established. Studies initiated with the discovery of feedback inhibition of the rate-limiting enzyme of endogen cholesterol biosynthesis, HMG-CoA, mediates feedback regulation of cholesterol synthesis by SREBP pathways (EBERLÉ *et al.* 2004; BROWN *et al*. 2002).

Cholesterol synthesis is a complex process with high-energy expenditure. Thus, the ability to control the synthesis to support the dietary intake is an advantage for the organism. As a response to cholesterol levels, organization is achieved by the transcriptional regulation of the gene coding HMG-CoA reductase (NELSON *et al*. 2008; SMALL & SHIPLEY 1974). The HMG-CoA reductase gene, plus over 20 other genes that encode enzymes regulating the cellular intake and synthesis of cholesterol and unsaturated fatty acids, are controlled by the transcription factor protein family named sterol regulator element binding proteins (SREBPs). These proteins are embedded in endoplasmic reticulum (ER) immediately after synthesis. Only the soluble amino terminal domains of SREBPs act as transcriptional activators. Yet this domain does not enter the nucleus when still a part of SREBP and therefore cannot participate in gene activation. In order to activate HMG-CoA reductase and the expression of other genes, the transcriptionally active domain is separated from SREBP by proteolytic cleavage (LEE *et al*. 2003). When cholesterol levels are high, SREBPs are inactive and kept in ER as a complex with another protein called SREBP- cleavage activating protein (SCAP). SCAP binds cholesterol and many other sterols, thus acting as a sterol sensor (EBERLÉ *et al*. 2004).

Recent studies identified two proteins of the ER, INSIG-1 and INSIG-2, with important functions in cholesterol metabolism. It has been shown that INSIGs inhibit the transition of the SCAP/SREBP complex from ER to Golgi working with sterols (YANG *et al*. 2002). INSIGs also negatively regulate HMG-CoA reductase transcription by inhibiting the activation of the transcription factor SREBP that is bound to the ER membrane (SEVER *et al*. 2003). Excess accumulation of cholesterol and triglycerides were shown in the livers of INSIG-1 and INSIG-2 knockout mice (ENGELKING *et al*. 2005). Therefore INSIG proteins mediate the regulation of cholesterol synthesis by sterol dependently binding to SCAP and HMG-CoA reductase (DONG *et al*. 2012).

Recently, the relations between the INSIG-SCAP-SREBP pathways have been investigated. HERBERT *et al*. (2006) reported that a single nucleotide polymorphism (SNP) located in the upstream region of the INSIG-2 gene, rs7566605, is associated with obesity (HERBERT *et al.* 2006). The relation between the INSIG-2 gene rs7566605 SNP and obesity has been confirmed in different studies on various populations (LYON *et al.* 2007; HALL *et al*. 2006; KUMAR *et al.* 2007; SMITH *et al*. 2007; WANG *et al*. 2008). In a study on Samoans, a population from the West Pacific with a high obesity rate, the INSIG-2 rs9308762 SNP located in the third intron was shown to be associated with body mass index (BMI) (P=0.024) and abdominal circumference (P=0.009) (DEKA *et al*. 2009). These researchers observed no association between either BMI or abdominal circumference and INSIG-2 gene rs7566605 SNP.

The ER proteins SCAP and INSIG may affect SREBP function in atherosclerosis and the pathogenesis of related diseases. No studies have investigated the associations between INSIG-2 and SCAP gene variations with atherosclerotic cardiovascular diseases. In this preliminary study, our aim was to determine the effect of two gene variants included in the Illumina Cardio-MetaboChip: the INSIG-2 gene rs9308762 and SCAP gene rs4858868 variations selected as critical for coronary artery disease risk and interaction with obesity parameters and lipid profiles in Turkish coronary artery patients.

Material and Methods

Description of the selected samples

In this study, the INSIG-2 rs9308762 and SCAP rs4858868 SNPs were evaluated in two groups comprised of 73 coronary artery disease (CAD) patients and 58 healthy controls. The control group was composed of healthy individuals with no history of hypertension and heart disease; the patient group was diagnosed with coronary heart disease attending the Department of Cardiology, Istanbul Medical Faculty, Istanbul University between 2013 and 2014. The patients with severe coronary vascular disease were documented by angiography. Angiographic inclusion criteria were: 50% stenosis of at least one major coronary vessel caused by atherosclerosis, and a vascular event, defined as myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery by-pass grafting. All subjects were subjected to full history with special emphasis on coronary risk factors including smoking, family history of coronary artery disease, diabetes mellitus, hyperlipidemia and hypertension. In the present study 45.8% of the individuals of the patient group were hypertensive, 43.1% had type 2 diabetes mellitus and 43.1% had left ventricular hypertrophy.

This study was arranged according to the "World Medical Association Declaration of Helsinki" and written consents were obtained from all participants. The study was approval by the Ethics Committees of the Istanbul Medical School, Istanbul University.

DNA isolation and genotyping

Genomic DNA was isolated from samples that were collected in sterile tubes containing EDTA by a commercial DNA isolation kit (Roche Diagnostics, GmbH, Mannheim, Germany). The INSIG-2 rs9308762 and SCAP rs4858868 SNPs were studied using the real-time PCR method. For the PCR reaction, 5µl of 100 ng/µl DNA samples were added to a mixture of 10.4 µl dH_2O, 1.6 µl $MgCl_2$ (25 mM), 2.0 µl LightCycler® FastStart HybProbe Reaction Mix and LightCycler® FastStart Enzyme mix (Roche Diagnostics GmbH, Mannheim, Germany), 1.0 µl flourescence tagged LightSNiP primer-probe set (TIB Molbiol GmbH, Berlin, Germany) that made the final volume of 20 µl. The amplification step was performed on the LightCycler® 480 device (Roche Diagnostics). Real-time PCR conditions included initial denaturation at 95°C for 10 minutes, 45 cycles of 95°C for 10 sec, 60°C for 10 sec and 72°C 15 sec.

Statistical analyses

The statistical analyses were performed in SPSS 20.0. Differences in mean lipid levels and other parameters were compared using Student's t and ANOVA tests. The INSIG-2 and SCAP genotype and allele comparisons, the comparisons of clinical and nonclinical parameters with alleles and for the comparison of the qualitative data such as con-

sistency to the Hardy-Weinberg Equilibrium, were evaluated using the chi square (χ^2) test. Allele frequencies were calculated with the gene counting method. In order to determine the relative risks, odds ratios and 95% confidence intervals were used and values of P<0.05 were considered significant.

Multivariate analysis was carried out by the linear logistic regression model. Estimation of the relative risk was determined by calculating odds ratios (OR) and confidence intervals (CI). For this analysis, the T allele of INSIG2 rs9308762 C>T SNP was used as a dependent variable and serum HDL-cholesterol level and body mass index were used as independent variables (Table 5).

Results

No significant differences between the patient and control groups were found in terms of mean age and gender (P>0.05) (Table 1). On the other hand, significant differences were observed when the systolic and diastolic blood pressures, serum HDL-cholesterol (HDL-C) values and smoking situations were compared between the CAD patients and controls. In the CAD group, fasting glucose, systolic (SBP) and diastolic (DBP) blood pressure and smoking (P<0.001) were at higher levels than in the control group, while HDL-C was lower (P=0.015). The risk threshold values for serum lipids and BMI between the CAD patient and control groups (total-cholesterol ≥5.18 mmol/l, triglyceride ≥1.70 mmol/l, HDL-cholesterol ≤0.90 mmol/l, LDL-C ≥3.36 mmol/l and BMI ≥27.5 kg/m^2) were not significantly different (P>0.05) (Table 1).

The genotype and allele distributions and their consistencies to HWE in the control and CAD patient groups are given in Table 2. While the control group was consistent to HWE regarding the SCAP rs4858868 G>A SNP (P>0.05), the patient group (P<0.05) was not. Only the distribution of SCAP rs4858868 SNP of the patient group showed a deviation from HWE, which may provide additional support for an association of the marker locus with the disease in question. However, deviation from

Table 1

The characteristic features of the study groups

	Groups		
	Control (n=58)	CAD Patients (n=73)	P values
Age (years)	57.86±7.31	60.19±12.18	0.207
Gender (Women/Men) (n)	23/35	23/50	0.332
BMI (kg/m^2)	25.45±3.11	25.57±3.80	0.860
Total-C (mmol/l)	4.83±1.23	5.27±1.41	0.067
TG (mmol/l)	1.53±0.65	1.65±0.81	0.342
HDL-C (mmol/l)	1.16±0.34	1.04±0.18	0.015
LDL-C (mmol/l)	3.07±1.09	3.29±1.12	0.275
VLDL-C (mmol/l)	0.70±0.30	0.74±0.34	0.464
SBP (mmHg)	117.04±8.42	139.03±32.80	0.001
DBP (mmHg)	69.06±8.12	85.69±17.98	0.001
Fasting blood glucose (mg/dl)	94.29±21.47	179.72±126.77	0.001
Smoking situation (%)	22.0%	56.9%	0.001
Family history of CAD (%)	30.4%	37.5%	0.551
Total-cholesterol ≥ 5.18 mmol/l (%)	39.3	45.2	0.500
TG ≥ 1.70 mmol/l (%)	33.3	41.7	0.340
HDL ≤ 0.90 mmol/l (%)	19.6	25.4	0.447
LDL ≥ 3.36 mmol/l (%)	39.3	41.4	0.808
BMI ≥ 27.5 kg/m^2 (%)	23.6	37.9	0.100

The age, serum lipid, BMI and blood pressure values in the table are given as $\bar{x}$+SD. The significance between the study groups was evaluated with Students't-test. CAD: Coronary artery disease, BMI: Body mass index, Total-C: Total Cholesterol, TG: Triglyceride, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, VLDL-C: VLDL-cholesterol, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, n: number of samples.

Table 2

Distribution of the INSIG2 and SCAP SNPs

	Study groups	
INSIG2 rs9308762 SNP		
rs9308762 Genotypes	Control (n=58)	CAD Patient (n=73)
CC	42 (72.4%)	55 (75.3%)
TT	3 (5.2%)	2 (2.7%)
CT	13 (22.4%)	16 (21.9%)
HWE	P>0.05	P>0.05
rs9308762 Alleles		
C	97 (83.62%)	126 (86.30%)
T	19 (16.38%)	20 (13.70%)
SCAP rs4858868 SNP		
rs4858868 Genotypes	Control (n=58)	CAD Patient (n=72)
GG	29 (50.0%)	31 (43.1%)
AA	6 (10.3%)	–
GA	23 (39.7%)	41 (56.9%)*
HWE	P>0.05	P<0.05
rs4858868 Alleles		
G	81 (69.83%)	103 (71.53%)
A	35 (30.17%)	41 (28.47%)

n – number of samples, HWE – Hardy-Weinberg Equilibrium.
* P<0.05 (chi-square=3.842, OR:1.402, 95% CI: 1.000-1.965).

HWE can also be due to the small sample size (GYORFFY *et al*. 2004; LEVECQUE *et al*. 2003). In contrast, both study groups' INSIG2 rs9308762 C>T SNP genotype distributions were found to be consistent to HWE (P>0.05). While the common C allele frequency was 83.62% and rare T allele frequency was 16.38% in the control group, in the CAD patient group it was 86.30% and 13.70%, respectively.

In the control group, while the common G allele of the SCAP rs4858868 G>A variation frequency was 69.83% and rare A allele frequency was 30.17%, in the CAD patient group it was 71.53% and 28.47%, respectively. In the CAD patient group, heterozygote GA genotype frequency was higher than the control group (39.7% vs. 56.9%; P<0.05, $\chi 2$=3.842, odds ratio (OR): 1.402, 95% confidential interval (CI): 1.000- 1.965), and the normal GG genotype was lower, however the difference was not statistically significant. In addition, since the SCAP rs4858868 rare AA genotype was not encountered in the CAD patient group, this allele was more frequent in the control group.

The investigation of the effects of the INSIG2 rs9308762 C>T genotype on serum lipid profile, blood pressure and BMI showed that INSIG rare T allele carriers have higher BMI values compared to homozygote CC carriers in the control group (P=0.017), yet the INSIG rs9308762 variant had no effect on serum lipid and blood pressure levels in the patient group (P>0.05). Moreover, the analyses performed with Student's t-test showed that the SCAP rs4858868 G>A genotype on serum lipid profile, blood pressure and BMI showed no relation in the study groups (P>0.05) (Table 3).

Statistical analyses showed no relationship with the INSIG2 rs9308762 C>T and SCAP rs4858868 G>A genotypes and the risk threshold values for serum lipids and BMI (total-cholesterol $\geq$ 5.18 mmol/l, triglyceride $\geq$ 1.70 mmol/l, HDL-C $\leq$ 0.90 mmol/l, LDL-C $\geq$ 3.36 mmol/l and BMI $\geq$ 27.5 kg/m^2) in the CAD patient group (P>0.05). On the other hand, in the control group a statistically significant difference was observed between the INSIG2 rs9308762 C>T variant and serum HDL-C levels being 0.90 mmol/l or less. In the control group, all individuals with HDL-C $\leq$ 0.90 mmol/l were homozy-

Table 3

The effects of the INSIG2 rs9308762 C>T and SCAP rs4858868 G>A genotypes on serum lipid profiles, blood pressure and BMI between study groups

Group	INSIG2 rs9308762		SCAP rs4858868	
	CC	TT/CT	GG	GA/AA
Control	n=42	n=16	n=29	n=29
BMI (kg/m^2)	24.84±2.93	27.07±3.08*	25.59±3.56	25.31±2.67
Total-C (mmol/l)	4.76±1.34	5.03±0.83	4.64±1.09	5.03±1.34
TG (mmol/l)	1.47±0.55	1.69±0.88	1.55±0.65	1.50±0.65
HDL-C (mmol/l)	1.18±0.39	1.12±0.09	1.18±0.44	1Ç14±0.19
LDL-C (mmol/l)	3.00±1.23	3.26±0.58	2.95±0.91	3.20±1.25
VLDL-C (mmol/l)	0.67±0.26	0.77±0.40	0.71±0.30	0.68±0.31
SBP (mmHg)	118.28±7.49	113.57±10.08	117.23±9.64	116.85±7.23
DBP (mmHg)	69.36±8.32	68.21±7.75	70.15±7.50	68.00±8.68
CAD Patient	n=55	n=18	n=31	n=41
BMI (kg/m^2)	25.85±3.83	24.67±3.67	25.85±3.70	25.47±3.90
Total-C (mmol/l)	5.38±1.53	4.93±0.86	5.26±1.29	5.32±1.49
TG (mmol/l)	1.60±0.83	1.81±0.76	1.68±0.91	1.63±0.75
HDL-C (mmol/l)	1.05±0.19	0.99±0.18	1.04±0.19	1.03±0.19
LDL-C (mmol/l)	3.36±1.21	3.11±0.82	3.22±0.77	3.39±1.33
VLDL-C (mmol/l)	0.71±0.33	0.83±0.35	0.73±0.34	0.75±0.34
SBP (mmHg)	141.30±35.06	132.22±34.39	136.29±28.05	140.88±36.62
DBP (mmHg)	86.76±18.29	82.50±17.17	84.19±16.44	86.50±19.32

The serum lipid, BMI and blood pressure values in the table are given as 0+SD. The significance was evaluated with the One-way ANOVA test between 3 group genotype comparisons; the 2 group allele comparisons were evaluated with Students't-test. CAD – Coronary artery disease, BMI – Body mass index, Total-C – Total Cholesterol, TG – Triglyceride, HDL-C – HDL-cholesterol, LDL-C – LDL-cholesterol, VLDL-C – VLDL-cholesterol, SBP – Systolic blood pressure, DBP – Diastolic blood pressure, n – number of samples. * P=0.017.

gote common CC allele carriers (Fisher's exact test, P=0.026) (Table 4).

The linear logistic regression analysis confirmed that the INSIG2 T allele is associated with increased BMI in control subjects. Regression analysis also revealed that the INSIG T allele could predict decreased mean serum HDL-cholesterol in CAD patients (Table 5).

Discussion

Cholesterol is a vital cell component and the precursor of steroid hormones, bile acids and oxysterols, which are important regulatory molecules in many metabolic pathways. Nevertheless, excessive accumulation around the vessels may obstruct the artery and lead to cardiovascular diseases (SMALL *et al*. 1974). Therefore, maintenance of the balance of cholesterol metabolism and understanding how it is managed remain the subjects of many studies.

Studies that started with the discovery of the feedback inhibition of the rate-limiting enzyme of the endogen cholesterol biosynthesis, HMG-CoA, demonstrated that the regulation of cholesterol synthesis is carried out by SREBP pathways (BROWN *et al*. 2002; EBERLÉ *et al*. 2004). SREBPs are the transcription factor family that regulate cell cholesterol homeostasis and play a role in fatty acid biosynthesis and its intracellular uptake. The members of this family are SREBP-1a, SREBP-1c, SREBP-2, and SREBP-2gc (WANG *et al*. 2004). SREBP-2 mainly controls the expressions of the

Table 4

The effects of the INSIG2 rs9308762 C>T and SCAP rs4858868 G>A genotypes on threshold metabolic risk assessments and patient characteristics in the study groups

Groups			INSIG2 rs9308762			SCAP rs4858868		
			CC	CT+TT	P value	GG	GA+AA	P value
Control								
	BMI	<27.5	32 (76.2%)	10 (23.8%)	0.300	19 (45.2%)	23 (54.8%)	0.304
		≥27.5	8 (61.5%)	5 (38.5%)		8 (61.5%)	5 (38.5%)	
	TC	<5.18	27 (79.4%)	7 (20.6%)	0.193	19 (55.9%)	15 (44.1%)	0.274
		5.18	14 (63.6%)	8 (36.4%)		9 (40.9%)	13 (59.1%)	
	TG	<1.70	29 (80.6%)	7 (19.4%)	0.124	20 (55.6%)	16 (44.4%)	0.441
		≥1.70	11 (61.1%)	7 (38.9%)		8 (44.4%)	10 (55.6%)	
	HDL	>0.90	30 (66.7%)	15 (33.3%)	0.026*	20 (44.4%)	25 (55.6%)	0.177
		≤0.90	11 (100%)	–		8 (72.7%)	3 (27.3%)	
	LDL	<3.36	26 (76.5%)	8 (23.5%)	0.494	17 (50.0%)	17 (50.0%)	1.000
		≥3.36	15 (68.2%)	7 (31.8%)		11 (50.0%)	11 (50.0%)	
CAD								
	BMI	<27.5	27 (75.0%)	9 (25.0%)	0.844	16 (45.7%)	19 (54.3%)	0.752
		≥27.5	17 (77.3%)	5 (22.7%)		11 (50.0%)	11 (50.0%)	
	TC	<5.18	29 (72.5%)	11 (27.5%)	0.535	18 (46.2%)	21 (53.8%)	0.564
		≥5.18	26 (78.8%)	7 (21.2%)		13 (39.4%)	20 (60.6%)	
	TG	<1.70	32 (76.2%)	10 (23.8%)	0.783	19 (45.2%)	23 (54.8%)	0.747
		≥1.70	22 (73.3%)	8 (26.7%)		12 (41.4%)	17 (58.6%)	
	HDL	>0.90	41 (77.4%)	12 (22.6%)	0.440	25 (47.2%)	28 (52.8%)	0.306
		≤0.90	13 (68.4%)	6 (31.6%)		6 (33.3%)	12 (66.7%)	
	LDL	<3.36	29 (70.7%)	12 (29.3%)	0.419	20 (50.0%)	20 (50.0%)	0.199
		≥3.36	23 (79.3%)	6 (20.7%)		10 (34.5%)	19 (65.5%)	

The values in the table are given as "n (%)". The significance was evaluated with the chi-square test. CAD – Coronary artery disease, BMI – Body mass index, TC – total-cholesterol, TG – Triglyceride, HDL-C – HDL-cholesterol, LDL-C – LDL-cholesterol. ***** P=0.026 (Fisher's exact test).

Table 5

Linear logistic regression analysis for the association between INSIG2 rs9308762 T allele, BMI and HDL-cholesterol level in the study groups (level of significance: P<0.05)

Groups	Dependent variable	Independent variables	P value	OR	95% CI for OR
Control	INSIG2 rs9308762 T allele	BMI	0.022	0.046	0.007-0.085
		HDL-cholesterol	0.908	(-)0.021	(-)0.377-0.335
CAD	INSIG2 rs9308762 T allele	BMI	0.193	(-)0.020	(-)0.049- 0.010
		HDL-cholesterol	0.045	(-)0.658	-1.300-(-)0.015

CAD – Coronary artery disease; BMI – Body mass index; OR – Odds ratio, CI – Confidence interval.

genes that function in cholesterolgenesis. Overexpression of SREBP-2 triggers the overexpression of all other enzymes that regulate cholesterol synthesis (SAKAKURA *et al*. 2001). SREBP-2gc is a curtailed form of SREBP-2 from the N-terminal, which is not affected by the feedback control of sterols, and regulates spermatogenic genes' transcriptions (WANG *et al*. 2004). As understood, SREBP-2 carries particular importance in the regulation of cholesterol metabolism, however other factors are also needed in the activation of the SREBP pathways. SCAP is a potent activator of the SREBP pathways in cholesterol synthesis. When cell cholesterol levels elevate, SCAP keeps SREBP in the endoplasmic reticulum, and in low sterol concentrations, SCAP accompanies the transition of SREBP from endoplasmic reticulum to Golgi. In Golgi, SREBP reaches its active form, nuclear SREBP (nSREBP), by two proteolytic cleavages. Later, nSREBPs translocate to the nucleus and there bind to sterol response elements in the promoter/enhancer regions of the target genes (SHIMOMURA *et al*. 1998).

Excessive accumulation of cholesterol and triglycerides have been reported in the livers of INSIG-1 and INSIG-2 knockout mice (ENGELKING *et al*. 2005). INSIG proteins sterol-dependently bind to SCAP and HMG-CoA reductase and thus mediate cholesterol synthesis. The INSIG2 rs9308762 SNP has been shown to be associated with BMI and abdominal circumference in Samoans, a population with high obesity rates in the West Pasific (DEKA *et al*. 2009). These researchers did not observe a relationship between the INSIG2 gene rs7566605 SNP and BMI and abdominal circumference, yet they reported an association with both BMI (P=0.024) and abdominal circumference (P=0.009) and the rs9308762 SNP located on the 3rd intron of the gene.

FORNAGE *et al*. (2010) studied 12 INSIG-2 SNPs with regard to their association with BMI, waist circumference and serum lipid levels and also investigated the interaction between coronary artery disease risk development with age prospectively in young adults (49.5% Black and 50.5% Caucasian participants) going back 20 years (FORNAGE *et al*. 2010). The INSIG-2 rs7566605 was not found to be associated with BMI or lipid metabolism according to both age and race, though rs1352083 and rs10185316 were found to be related to HDL-C in Caucasians declining with age (P<0.05). A similar tendency was also observed in Black participants whose BMI values were below 25 kg/m^2.

There are a limited number of studies investigating the association between the SCAP gene and metabolism or plasma lipids. LE HELLARD *et al*. (2009), in their study on a German population, reported a relationship between the SCAP rs12490383 SNP and BMI values. FIEGENBAUM *et al*. (2005) in their study on 146 European hypercholesterolemic patients, evaluated the effects of the SNPs of SREBF-1a, SREBF-2 and SCAP genes in response to the lipid-lowering drug simvastatin (20 mg/day simvastatin treatment for 6 months). They measured the plasma lipid and lipoprotein levels of the participants before and during the study. As a result of this study, the SCAP 2386 A>G (rs12487736) SNP, compared to homozygote 2386-AA genotypes, had a greater decrease in their total cholesterol (TC) (P=0.007), and a similar trend was observed in their LDL-C levels (P=0.088). Additionally, an interaction between plasma triglyceride (TG) levels and response to treatment was observed; the decrease in their TG levels were 7.9% in the 2386-G allele carriers while homozygote 2386-AA carriers showed a 8.7% increase (P=0.016) (FIEGENBAUM *et al*. 2005). ZAVATTARI *et al*. (2010) in their study on 747 Sardinian obese children and adolescents, suggested that the rs7566605 G>C SNP that is located 10kb upstream in the INSIG-2 gene may play a role in obesity-related metabolic complications (ZAVATTARI *et al*. 2010). The INSIG-2 rs7566605 G>C allele frequencies were found to be similar between the patient and control groups. Mean glucose and insulin levels and HOMA-IR values (insulin resistance) were higher in the CC genotype carriers than CG and GG carriers (ZAVATTARI *et al*. 2010). REINEHR *et al*. (2009) investigated the effect of lifestyle differences on weight gain in 280 overweight children from Germany with 2 different SNPs (INSIG2: rs7566605, FTO: rs9939609). In the statistical analysis of this study that was performed with an adjusted by age and gender according to BMI, the INSIG-2 CC and FTO AA genotype combination was found to be associated with the lowest degree of weight loss (REINEHR *et al*. 2009). WANG et al. in their study on Chinese children and adolescents, investigating the effect of the INSIG2 rs7566605 variant on the obesity related phenotype, the homozygote CC carrier obese children had higher triglyceride levels than the GG and GC genotype carriers (P=0.022) (WANG *et al*. 2008).

In the present study, the INSIG2 rs9308762 C>T variant distributions were similar among study groups (P>0.05), while the SCAP rs4858868 G>A heterozygote GA genotype frequency was higher compared to the control group (P=0.05). In addition, the SCAP rs4858868 rare AA genotype was not encountered in the CAD patient group. When the INSIG-2 rs9308762 C>T genotypes were evaluated in terms of serum lipid profiles, blood pressures and BMI values, there was no effect observed in the CAD patient group (P>0.05), while in the control group the rare T allele carriers had

higher BMI values than the homozygote CC genotype carriers (P=0.017). No association was observed between the serum lipid and blood pressure levels in the healthy control group with the INSIG rs9308762 variant.

The INSIG rs9308762 and BMI association is compatible with the BMI and abdominal circumference relation found in the study conducted on the Samoan population (DEKA *et al.* 2009). However, there was no effect of the SCAP rs4858868 G>A SNP on the serum lipid profiles, blood pressures and BMIs of the CAD patients (P>0.05). In the CAD patient group, while there was no effect of the INSIG-2 rs9308762 C>T and SCAP rs4858868 G>A SNPs on the risk threshold values for serum lipids and BMI (total-cholesterol ≥ 5.18 mmol/l, triglyceride ≥ 1.70 mmol/l, HDL-C ≤ 0.90 mmol/l, LDL-C ≥ 3.36 mmol/l and BMI ≥ 27.5 kg/m^2) (P>0.05), in the healthy controls a statistically significant difference was observed between the INSIG2 rs9308762 C>T common CC homozygote genotype and serum HDL-C values of 0.90 mmol/l or below (P=0.026).

Consequently, these findings suggest that the INSIG2 rs9308762 C>T SNP may be associated with obesity serum lipoprotein levels. In addition, the results suggest that the SCAP rs4858868 G>A SNP might be associated with an increased CAD risk independently of other metabolic parameters. Furthermore, our findings have supported that these gene variants that might be contributing to the development of atherosclerosis related complications.

Acknowledgements

This study was funded by the Scientific Research Projects Unit of Istanbul University. Project No: 37401.

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