Turk Gogus Kalp Dama 2015;23(4):637-642
doi: 10.5606/tgkdc.dergisi.2015.11169

Original Article / Özgün Makale

Adiponectin and leptin polymorphisms in patients with coronary artery disease

Koroner arter hastalarında adiponektin ve leptin polimorfizmleri

Sefa Şenol,[1] Hayrettin Tekümit,[2] Murat Kara,[3] Kürşat Kargün,[3] Mustafa Yıldız[4]

Institution where the research was done:
Elazığ Training and Research Hospital, Elazığ, Turkey

Author Affiliations:
[1]Department of Cardiovascular Surgery, Elazığ Training and Research Hospital, Elazığ, Turkey
[2]Department of Cardiovascular Surgery, Medical Faculty of Bezmialem Vakıf University, İstanbul, Turkey
Departments of [3]Genetic Medicine, [4]Emergency Medicine, Medical Faculty of Elazığ University, Elazığ, Turkey

ABSTRACT

Background: In this study, adiponectin and leptin gene polymorphism were analyzed in patients with planned coronary artery bypass graft (CABG) surgeries.

Methods: Between January 2012 and April 2013, 110 patients in the study group (78 males, 32 females; mean age 61.3±7.8 years; range 47 to 76 years) were scheduled for CABG surgery for coronary artery disease (CAD) and 96 healthy subjects without CAD in the control group (68 males, 28 females; mean age 59.4±8.3 years; range 46 to 78 years) were included in the study.

Results: Adiponectin rs2241766 gene polymorphism and allele frequency were statistically significantly lower in the study group ($p<0.05$). Leptin receptor rs1137101 gene polymorphism and allele frequency were statistically significantly higher in the study group, compared to the controls ($p<0.05$). The rs7799039 gene polymorphism of leptin was statistically significantly higher in the control group ($p<0.05$), while there was no statistically significant difference in the allele frequency between the groups ($p>0.05$). Gene polymorphisms of adiponectin at rs1501299 and their allele frequencies were similar between the study and the control groups ($p>0.05$).

Conclusion: Our study results showed that gene polymorphism of adiponectin at rs2241766 decreased the risk of CAD, while gene polymorphism of leptin receptor rs1137101 and rs7799039 increased the risk. By demonstrating the biochemical consequences of adiponectin and leptin polymorphisms, this study may, together with larger and more comprehensive studies, serve as a reference concerning these risks associated with these polymorphisms and the benefits of prophylaxis in CAD.

Keywords: Adiponectin; coronary artery disease; leptin; polymorphism.

ÖZ

Amaç: Bu çalışmada, koroner arter baypas greft (KABG) cerrahisi yapılacak hastalarda adiponektin ve leptin gen polimorfizmleri analiz edildi.

Çalışma planı: Ocak 2012 - Nisan 2013 tarihleri arasında çalışma grubunda koroner arter hastalığı (KAH) nedeniyle KABG yapılması planlanan toplam 110 hasta (78 erkek, 32 kadın; ort. yaş 61.3±7.8 yıl; dağılım 47-76 yıl) ve kontrol grubunda KAH olmayan 96 sağlıklı birey (68 erkek, 28 kadın; ort. yaş 59.4±8.3 yıl; dağılım 46-78 yıl) çalışmaya dahil edildi.

Bulgular: Adiponektin rs2241766 gen polimorfizmi ve allel görülme sıklığı, çalışma grubunda istatistiksel olarak anlamlı düzeyde daha düşüktü ($p<0.05$). Leptin reseptör rs1137101 gen polimorfizmi ve allel görülme sıklığı, kontrollere kıyasla, çalışma grubunda istatistiksel olarak anlamlı düzeyde daha yüksekti ($p<0.05$). Leptin rs7799039 gen polimorfizmi, kontrol grubunda istatistiksel olarak anlamlı düzeyde daha yüksek iken ($p<0.05$), allel görülme sıklığı açısından gruplar arasında istatistiksel olarak anlamlı bir fark yoktu ($p>0.05$). Adiponektik rs1501299 gen polimorfizmleri ve allel görülme sıklıkları, çalışma ve kontrol grupları arasında benzerdi ($p>0.05$).

Sonuç: Çalışma bulgularımız, adiponektin rs2241766 gen polimorfizminin KAH riskini azalttığını, ancak leptin reseptörü rs1137101 ve rs7799039 gen polimorfizminin riski artırdığını gösterdi. Adiponektin ve leptin polimorfizmlerinin biyokimya sonuçlarını gösteren bu çalışma, daha geniş ölçekli ve daha kapsamlı çalışmalar ile birlikte, bu polimorfizmlere bağlı riskler ve KAH'ta profilaksinin yararına ilişkin bir referans olabilir.

Anahtar sözcükler: Adiponektin; koroner arter hastalığı; leptin; polimorfizm.

Available online at
www.tgkdc.dergisi.org
doi: 10.5606/tgkdc.dergisi.2015.11169
QR (Quick Response) Code

Received: November 16, 2014 *Accepted:* April 08, 2015

Correspondence: Sefa Şenol, M.D. Elazığ Eğitim ve Araştırma Hastanesi Kalp ve Damar Cerrahisi Kliniği, 23200 Elazığ, Turkey.
Tel: +90 424 - 237 49 43 e-mail: ssenol2012@gmail.com

Etiology of coronary artery disease (CAD) is multi-factorial including physiological, environmental, and genetic aspects.[1] As the elderly population increases, a higher number of older patients with ischemic heart disease has been undergoing coronary artery bypass grafting (CABG) in developing countries.[2]

Although preoperative use of non-invasive tests is critical to identify cardiac risks, predictive values of these tests are approximately 25%.[3] In the United States (USA), 40 million patients have surgery annually with an estimated cost of 450 trillion dollars. In addition, it has been estimated that the number of surgeries will increase by 25%, related cost will increase by 50%, and cardiac, cerebral, and renal complications related to atherosclerosis will increase by 100% by 2020.[3] Investigation of the genetic factors is also important to prevent increasing treatment costs and surgical complications and to take precautions for patients with CAD.[3]

Adiponectin is mainly released by white adipocytes.[4] It contributes to the phosphorylation of nitric oxide synthase in endothelial cells (eNOS) and increases the expression/activity of eNOS. It also stimulates production of interleukin 10 (IL-10), an anti-inflammatory cytokine found in macrophages, and increases the matrix metalloproteinase tissue inhibitor-1 production; and, hence, it plays a major role in the stabilization of the atherosclerotic plaques.[4] Adiponectin has been shown to decrease tumor necrosis factor alpha (TNF-α) secretion from monocyte macrophages. It has also been shown to inhibit macrophage foam cell formation by downregulation of the scavenger receptor A (SR-A) expression and to decrease intracellular cholesteryl ester content of the macrophages.[4,5] In addition, adiponectin increases nitric oxide (NO) production in the endothelial cells, stimulating angiogenesis.[6,7] It has been suggested to prevent endothelial dysfunction and atherosclerosis thanks to its anti-inflammatory, antioxidant, and vasodilator effects, and CAD has been linked with hypoadiponectinemia.[4,8] Some polymorphisms of adiponectin were not found to be associated with CAD, while some others were reported to decrease CAD.[9]

Leptin, a peptide hormone resembling cytokines, contains 167 amino acids.[10] It plays a key role in the etiology of obesity and has been suggested that it may contribute to the development of hypertension and atherosclerosis in obese patients.[11]

Low baseline serum leptin levels have been considered as an indicator of future cardiovascular events and death in stable cardiovascular patients.[10] However, several studies have shown a strong relationship between high serum leptin levels and atherosclerosis.[10,12] In addition, it has been suggested that leptin receptor gene variations may be independently associated with premature atherosclerosis and some risk factors.[11]

In this study, we aimed to analyze adiponectin rs1501299 and rs2241766 and leptin receptor rs1137101 and rs7799039 gene polymorphisms in patients with planned CABG surgeries.

PATIENTS AND METHODS

Between January 2012 and April 2013, 110 patients in the study group (78 males, 32 females; mean age 61.3±7.8 years; range 47 to 76 years) were scheduled for CABG surgery for CAD in our clinic. The control group consisted of 96 healthy subjects (68 males, 28 females; mean age 59.4±8.3 years; range 46 to 78 years) without CAD. This is a prospective, case-control study in which patients undergoing CABG were compared to those without CAD in a non-random fashion. Exclusion criteria were as follows: myocardial infarction in the previous month, significant valvular problems, or congenital heart disease, cardiomyopathy, renal or liver dysfunction, or any known systemic disease, and acute coronary syndrome on admission. The main reason for the exclusion of acute myocardial lesions was based on the role of adiponectin in the stabilization of atherosclerotic plaques.[4]

The study protocol was approved by the institutional ethics committee. An informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Helsinki Declaration.

Demographic data of the patients, physical examination findings, and cardiovascular risk factors were recorded. The patients with a fasting blood glucose level >126 mg/dL and those ones on oral anti-diabetics or insulin treatment were regarded as diabetic patients. The patients with a systolic blood pressure of ≥140 mmHg and a diastolic blood pressure of ≥90 mmHg, and those on an antihypertensive treatment were regarded as hypertensive patients. The blood samples were obtained for routine hematological and biochemical tests following a 12-hours fasting period. Coronary angiography was defined as the qualitative and quantitative diagnostic gold standard for CABG patients before planning surgery.

A venous blood sample of 2-3 mL was drawn from all participants and put into tubes with ethylenediamine tetraacetic acid (EDTA). Deoxyribonucleic acid (DNA)

Table 1. Demographic and clinical characteristics of the patient and control groups

	CABG group			Control group			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			61.3±7.8			59.4±8.3	0.32
Gender							0.62
Female	32			28			
Male	78			68			
Hypertension		41.8			5		0.001
Fasting glucose (mg/dL)			108.9±20.4			88±18.4	0.03
Total cholesterol (mg/dL)			176±48			175±39	0.78
Triglyceride (mg/dL)			143±91			137±75	0.46
High-density lipoprotein cholesterol (mg/dL)			42±17			41±10	0.06
Low-density lipoprotein cholesterol (mg/dL)			125±40			116±43	0.08
Urea (mg/dL)			35.3±8.1			34.9±7.9	0.67
Creatinine (mg/dL)			0.84±0.19			0.81±0.26	0.51
Diabetes mellitus		42.5			8.75		0.001
Body mass index (kg/m^2)			29.5±2.1			26.5±4.3	0.48
Family history		25.2			23.4		0.72

CABG: Coronary artery bypass grafting; SD: Standard deviation.

isolations were done in Medical Genetics Laboratory using the DNA isolation kit (PureLink™ genomic DNA kits, Invitrogen, Carlsbad, CA 92008 USA). Isolated DNAs were stored at -20 °C until the analyses were performed. Genotyping was performed with Applied Biosystems™ Real-Time PCR Instruments StepOne Plus Real Time Polymerase Chain Reaction (PCR) equipment (Applied Biosystems, Foster City, CA, USA).[13]

Statistical analysis

Statistical analysis was performed using the SPSS version 11.5 software (SPSS Inc., Chicago, IL, USA). Differences in genotype distribution and consistency with Hardy-Weinberg equilibrium were tested by chi-square test. The test was also used to analyze the categorical variables. Intergroup comparisons were done with one-way variance analysis (One-way ANOVA) test. T test and Mann-Whitney U test were performed to compare intra-group variables. The results were analyzed with a confidence interval of 95% and a *p* value of <0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the groups are shown in Table 1. Age, sex, level of total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol, body mass index (BMI), and medical family history were similar between the groups

Table 2. Adiponectin genotype distribution

	CABG group		Control group		
	n	%	n	%	*p*
rs1501299					
GG	72	65.5	60	62.5	
GT	32	29.1	30	31.3	0.4010
TT	6	5.5	6	6.3	
Total	110	100	96	100	
rs2241766					
TT	60	54.5	6	6.2	
TG	20	18.2	24	26.0	0.0001
GG	30	27.3	66	67.7	
Total	110	100	96	99.9	

CABG: Coronary artery bypass grafting.

Table 3. Leptin genotype distribution

	CABG group		Control group		
	n	%	n	%	*p*
rs1137101					
AA	76	69.1	48	50	
AG	24	21.8	32	33.3	0.0142
GG	10	9.1	16	16.7	
Total	110	100	96	100	
rs2241766					
GG	34	30.9	26	27.1	
GA	36	32.7	56	58.3	0.0003
AA	40	36.4	14	14.6	
Total	110	100	96	100	

CABG: Coronary artery bypass grafting.

Table 4. Allele frequency for adiponectin

	CABG group		Control group		
	n	%	n	%	*p*/OR
rs1501299					
Allel					
G	176	80	150	78.1	0.6404/0.89
T	44	20	42	21.9	
rs2241766					
Allel					
T	140	63.6	37	19.3	0.0001/0.13
G	80	36.4	155	80.7	

CABG: Coronary artery bypass grafting; OR: Odss ratio.

Table 5. Allele frequency for leptin

	CABG group		Control group		
	n	%	n	%	*p*/OR
rs1137101					
Allel					
A	176	80	128	66.7	0.021/0.50
G	44	20	64	33.3	
rs7799039					
Allel					
G	104	47.3	108	56.2	0.068/1.43
A	116	52.7	84	43.8	

CABG: Coronary artery bypass grafting; OR: Odss ratio.

(p>0.05). However, the CABG group had higher fasting glucose levels as well as higher rates for diabetes and hypertension, compared to the control group (p<0.05). The genotype distributions of adiponectin in the study and the control groups are shown in Table 2. There was no significant difference in adiponectin rs1501299 gene polymorphism between the study group and controls (p=0.4010).

For rs2241766, another region of adiponectin, homozygous TT polymorphism was significantly higher in the CABG group, compared to the healthy controls. The mutant region frequency was found significantly higher in the control group, compared to the study group (p=0.0001).

The distribution of leptin genotypes in the study and the control groups is presented in Table 3. There was a significant difference in leptin receptor rs1137101 (p=0.0142) and rs7799039 (p=0.0003) gene polymorphisms between the study and control groups.

The allele frequency for adiponectin is shown in Table 4. The study and the control groups had similar allele frequencies of adiponectin at rs1501299 (p=0.95). T allele frequency of adiponectin at rs2241766 was seen in 63.6% patients in the study group and in 19.3% patients in the control group. In addition, G allele frequency was seen in 36.4% patients in the study group and in 80.7% in the control group. These findings showed that T allele frequency of adiponectin at rs2241766 was higher, whereas G allele frequency was lower in the study group, compared to the controls (p=0.0001).

The allele frequencies of leptin are shown in Table 5. A and G allele frequencies of leptin at rs7799039 did not show any significant differences between the study and the control groups (p=0.068). However, leptin receptor rs1137101 allele frequencies were significantly higher in the study group, compared to the controls (p=0.021).

DISCUSSION

Although Zhang et al.[14] reported that further, larger studies are required, their meta-analysis showed that rs2241766G allele of adiponectin increased the risk of cardiovascular diseases, whereas rs1501299T allele decreased the risk. In contrast to aforementioned study, our study showed that rs2241766 G allele of adiponectin decreased the risk for CAD. However, we found no relationship between rs1501299 T allele of adiponectin and CAD.

Another study performed in China reported that adiponectin rs2241766 polymorphism was no associated with CAD, while adiponectin rs1501299 polymorphism decreased the risk of CAD.[8] However, in our study, we found that adiponectin rs2241766 G allele decreased the risk of CAD. However, adiponectin rs1501299 T allele was not associated with CAD in our study.

Another meta-analysis showed that adiponectin rs2241766 was not associated with CAD. However, adiponectin rs1501299 polymorphism was associated with the disease, exerting a protective effect on CAD. It was also reported that this polymorphism decreased CAD risk in Caucasians; however, it increased the risk for disease in Asians.[15] In our study, we found different results from those aforementioned meta-analyses, as we compared CAD patients scheduled for CABG with the healthy subjects. Based on our study results, the presence of adiponectin rs2241766 polymorphism decreased the risk of CAD. However, we did found no association between the presence of gene polymorphism of adiponectin at rs1501299 and CAD.

Some authors have suggested that leptin plays an important role in the etiology of obesity and there is a number of studies investigating the role of leptin on the development of hypertension and atherosclerosis in this

patient population.[11,12] Saukko et al.[12] emphasized that leptin receptor gene variations might be independently associated with premature atherosclerosis and some risk factors. In consistent with these findings, we found that leptin receptor Gln223Arg (rs1137101) gene polymorphism and allele frequency were significantly higher in the study group, compared to the controls.

Although a number of studies have, to date, investigated the possible relation of leptin level and CAD, leptin polymorphisms have not been investigated as much as the polymorphisms of adiponectin. Several studies showed that leptin levels were predictive for cardiovascular events.[16,17] In our study, we found similar results: there was a significant difference in leptin rs7799039 and leptin receptor rs1137101 gene polymorphism between the study and control groups.

Furthermore, various studies have shown that leptin levels are associated with obesity and insulin levels.[18] In our study, however, there were no significant differences in the BMI values between the study and control groups. On the other hand, we found a significant difference in leptin rs7799039 and leptin receptor rs1137101 gene polymorphisms. This suggests that leptin gene polymorphisms may exert their effects through various biochemical mechanisms other than obesity. We also observed a significant difference in the incidence of diabetes between the two groups. We believe that further studies investigating these polymorphisms together with serum insulin and leptin levels in diabetic and non-diabetic CAD patients would enable us to obtain more conclusive results.

Of note, the statistical non-significance of adiponectin rs1501299 gene polymorphisms in our study, which is inconsistent with the current literature data, can be attributed to the genetic differences of CAD patients in the Turkish population.

On the other hand, small sample size was the limitation to this study. We believe that a larger sample size may increase the statistical power of such studies.

In conclusion, gene polymorphism of adiponectin at rs2241766 decreased the risk of CAD, while gene polymorphism of leptin receptor rs1137101 and rs7799039 increased the risk in our study. By demonstrating the biochemical consequences of adiponectin and leptin polymorphisms, this study may, together with larger and more comprehensive studies, serve as a reference concerning these risks associated with these polymorphisms and the benefits of prophylaxis in CAD.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Ellsworth DL, Sholinsky P, Jaquish C, Fabsitz RR, Manolio TA. Coronary heart disease. At the interface of molecular genetics and preventive medicine. Am J Prev Med 1999;16:122-33.
2. Polat A, Tekümit H, Cenal AR, Tataroğlu C, Uzun K, Mert B, et al. Mid-term results of coronary artery bypass graft surgery in patients 70 years of age or older.Turk Gogus Kalp Dama 2012;20: 467-73.
3. Senol S, Es MU, Gokmen G, Ercin O, Tuylu BA, Kargun K. Genetic polymorphisms in preoperative myocardial infarction.v Asian Cardiovasc Thorac Ann 2015;23:389-93.
4. Ouchi N, Walsh K. Adiponectin as an anti-inflammatory factor. Clin Chim Acta 2007;380:24-30.
5. Yang H, Zhang R, Mu H, Li M, Yao Q, Chen C. Adiponectin promotes endothelial cell differentiation from human peripheral CD14+ monocytes in vitro. J Cell Mol Med 2006;10:459-69.
6. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. J Biol Chem 2003;278:45021-6.
7. Knobler H, Benderly M, Boyko V, Behar S, Matas Z, Rubinstein A, et al. Adiponectin and the development of diabetes in patients with coronary artery disease and impaired fasting glucose. Eur J Endocrinol 2006;154:87-92.
8. Liu F, He Z, Deng S, Zhang H, Li N, Xu J. Association of adiponectin gene polymorphisms with the risk of ischemic stroke in a Chinese Han population. Mol Biol Rep 2011;38:1983-8.
9. Dubey L, Hesong Z. Role of leptin in atherogenesis. Exp Clin Cardiol 2006;11:269-75.
10. Ku IA, Farzaneh-Far R, Vittinghoff E, Zhang MH, Na B, Whooley MA. Association of low leptin with cardiovascular events and mortality in patients with stable coronary artery disease: the Heart and Soul Study. Atherosclerosis 2011;217:503-8.
11. Hasan-Ali H, Abd El-Mottaleb NA, Hamed HB, Abd-Elsayed A. Serum adiponectin and leptin as predictors of the presence and degree of coronary atherosclerosis. Coron Artery Dis 2011;22:264-9.
12. Saukko M, Kesäniemi YA, Ukkola O. Leptin receptor Lys109Arg and Gln223Arg polymorphisms are associated with early atherosclerosis. Metab Syndr Relat Disord 2010;8:425-30.
13. Senol S, Akar I, Kargün K, Bayram A, Kara M, Secen Ö. Endothelin-1 gene polymorphism in preoperative myocardial infarction with/or without coronary artery bypass graft. Int J Hum Genet 2014;14:183-7.

14. Zhang H, Mo X, Hao Y, Gu D. Association between polymorphisms in the adiponectin gene and cardiovascular disease: a meta-analysis. BMC Med Genet 2012;13:40.
15. Yang Y, Zhang F, Ding R, Wang Y, Lei H, Hu D. Association of ADIPOQ gene polymorphisms and coronary artery disease risk: a meta-analysis based on 12 465 subjects. Thromb Res 2012;130:58-64.
16. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104:3052-6.
17. Wolk R, Berger P, Lennon RJ, Brilakis ES, Davison DE, Somers VK. Association between plasma adiponectin levels and unstable coronary syndromes. Eur Heart J 2007;28:292-8.
18. Nalini D, Karthick R, Shirin V, Manohar G, Malathi R. "Role of the adipocyte hormone leptin in cardiovascular diseases - a study from Chennai based Population". Thromb J 2015;13:12.