Multi-Drug Resistance-1 [*MDR1*, P-gp, *ABCB1*] Gene Polymorphism in Patients with Abdominal Aortic Aneurysm and Their Family Members

Abdominal Aort Anevrizması Bulunan Hastalarda ve Aile Üyelerinde Multi-Drug Resistance-1 [*MDR1*, P-gp, *ABCB1*] Geni Polimorfizmi

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ABSTRACT

Objective: The aim of this study was to investigate the relationship between the 3435C>T polymorphism in the multi-drug resistance-1 gene [MDR1, P-gp, ABCB1] and the development of abdominal aortic aneurysm (AAA).

Material and Methods: This study included 75 patients with AAA (Group I), 75 individuals who were first-degree relatives of these patients but did not have AAA diagnosis (Group II), and 75 healthy volunteers whose abdominal aortic diameters were in the normal range (Group III). Genomic DNAs of these individuals were obtained from the peripheral blood. The MDR1:c.3435C>T polymorphism [CC (Wild Type), CT (Heterozygous) and TT (Homozygous) genotypes] was investigated by using the real-time polymerase chain reaction technique (real-time PCR).

Results: MDR1 TT genotype was significantly more frequent in Groups I and II compared to Group III (33.3% and 38.6% vs. 8%, respectively, $p<0.001$). *MDR1* CT genotype was also more frequent in Group I and Group II in comparison with Group III (44% and 42.6% vs 25.3%, respectively, $p<0.01$).

Conclusion: The presence of an association between the *MDR1* gene polymorphism and the development of AAA suggests that this gene may trigger the pathological mechanisms that play a role in the development of AAA. Additionally, patients with AAA and their first-degree relatives possess similar genetic characteristics in terms of the *MDR1* gene polymorphism. Therefore, *MDR1* gene polymorphism may be a risk factor for AAA in individuals with a positive family history of AAA.

Key Words: Abdominal aortic aneurysm, MDR1 gene polymorphism

ÖZ

Amaç: Bu çalışmanın amacı Multi-drug resistance-1 [MDR1, P-gp, ABCB1] genindeki 3435C>T polimorfizmi ile abdominal aort anevrizması (AAA) gelişimi arasındaki ilişkinin incelenmesidir.

Gereç ve Yöntemler: AAA tanısı olan 75 hasta (Grup I), bu hastaların AAA tanısı olmayan birinci derece akrabalarından oluşan 75 gönüllü (Grup II) ve aort çapları normal olan ve akrabaları olmayan 75 sağlıklı gönüllü (Grup III) çalışmaya alındı. Periferik kandan olgulara ait genomik DNA'lar elde edildi. MDR1:c.3435C>T polimorfizmi [CC (Doğal genotip), CT (Heterozigot) ve TT (Homozigot) genotipler] eş zamanlı polimeraz zincir reaksiyonu (real-time PCR) tekniği kullanılarak araştırıldı.

Bulgular: *MDR1* TT genotipinin Grup I ve Grup II'de kontrol grubuna göre anlamlı derecede fazla olduğu görüldü (sırasıyla %33,3 ve %38,6'e karşı %8, $p<0,001$). *MDR1* CT genotipinin de Grup I ve Grup II'de kontrol grubuna göre anlamlı derecede fazla olduğu görüldü (sırasıyla %44 ve %42,6'a karşı %25,3, $p<0,01$).

Sonuç: *MDR1* gen polimorfizminin AAA gelişimi ile bir ilişki göstermesi, bu genin AAA gelişiminde rol alan patolojik mekanizmaları tetikleyebileceğini düşündürmektedir. Ek olarak, AAA'lı bireyler ve birinci derece yakınları *MDR1* gen polimorfizmi açısından benzer genetik özellikler taşımaktadır. *MDR1* gen polimorfizmi AAA açısından aile hikayesi bulunan bireylerde AAA gelişimi için bir risk faktörü olabilir.

Anahtar Sözcükler: Abdominal aort anevrizması, MDR1 gen polimorfizmi

INTRODUCTION

Aneurysm is an abnormal and irreversible increase in the diameter of aorta in any segment relative to its normal diameter according to age and body surface area of the patient. Abdominal aortic aneurysm (AAA) is described as an 1.5-fold increase in the transverse diameter of the abdominal aorta (1). AAA develops with the aneurysmatic dilatation of the subdiaphragmatic aorta.

MDR1 is a gene with 29 exons localized in chromosome 7q21.1 and corresponds to a 4916-nt RNA. The *MDR1*-encoded P-glycoprotein contains 1280 amino acids and is a member of the ATP-dependent membrane transport family. Its molecular weight is 141,462 Daltons (2-5).

Previous studies have investigated the effects of gene mutations and genetic predisposition on AAA. More frequent AAA occurrence in congenital diseases such as Ehler-Danlos and Marfan syndromes suggests that AAA may be a result of congenital metabolic disorders. Furthermore, significant results have been obtained for the association of the polymorphism in the *MDR1* gene with the development of AAA by causing drug resistance along with resistant hypertension, with effects on cholesterol and cell proliferation, with disruption of the wall structure with pro-inflammatory processes, and with oxidative stress and detoxification (6).

Our aim was to investigate the relationship between AAA and the 3435C>T polymorphism in the *MDR1* gene, which was believed to be a risk factor for the development of AAA. We have investigated the rs1045642:C>T polymorphism (NG_011513:c.3435C>T) in order to describe the genetic basis of AAA development by including the first-degree relatives in this prospectively planned study for the first time in Turkey.

MATERIALS and METHODS

This study was conducted between September 2009 and July 2011. The study included the patients who were diagnosed with abdominal aortic aneurysm (AAA) clinically and through contrast-enhanced computerized thoracoabdominal tomographic imaging (Group I), their first-degree relatives who did not have AAA (Group II), and healthy individuals that matched the patients for age and gender (Group III). Of the patients (Group I), 61 were treated surgically (8 had emergency surgery). Patients with other arterial aneurysms were excluded. All subjects were evaluated for the presence of factors that could contribute to development of AAA such as hypertension (HT), hyperlipidemia (HL), coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), increased CRP levels, and smoking. Lipid profiles of the 8 patients who had emergency surgical treatment were determined on postoperative day 1. The subjects were evaluated for the presence of CAD by means of non-invasive tests (medical history, physical examination, ECG, cardiovascular stress testing, echocardiography, and nuclear medicine examinations). All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all subjects included in the study

DNA Isolation

A 5 mL blood sample from each subject was put in a 15 mL centrifuge tube containing 1 mL EDTA (Sigma E-5134, USA) (2%) and stored at -20 °C until DNA was isolated. DNA isolation was done with a DNA isolation kit (NucleoSpin Blood, Macherey-Nagel, Germany). The method involves the denaturation of all structures except DNA material in the white blood cells after which the remaining genomic DNA was isolated according to kit instructions.

Determination of *MDR1*:c.3435C>T Polymorphism with Real Time PCR

Genotypes of subjects in terms of the 3435C>T polymorphism in the *MDR1* gene were determined with real-time polymerase chain reaction (real-time PCR) using the genomic DNAs in Light Cycler System (Roche Molecular Diagnostics, USA). The reverse hybridization method was also used simultaneously.

Statistical Analysis

SPSS 13.0 software was used in the statistical analysis of the data. A p value of <0.001was considered as statistically significant. The software also calculated the odds ratio (OR) and 95% confidence interval (CI). The statistical power of this study was found to be 89%, with 75 patients, a standard deviation of 15.8, and differences of 7.33 (means x standard deviation). The association between AAA and predisposing factors and the MDR1 gene mutation was evaluated with the chi-square test. The association between AAA and genetic variation was also evaluated by multivariate regression analysis.

RESULTS

The demographic characteristics of the subjects are presented in Table I. The factors that predispose to the development of AAA were similar in the three groups (Table I).

In the assessment of aortic diameters of the subjects, the mean aortic diameter was 53.23 ±9.75 mm in Group I, 24.87 ±3.79 mm in Group II, and 25.97 ±3.45 mm in Group III (p<0.001). The difference between the mean aortic diameter of Group I and those of Group II and Group III was significant (Table 2).

The variations in the MDR1 gene from the study and control groups were compared as CC (Wild Type), CT (Heterozygous), and TT (Homozygous) genotypes. In Group I, CC genotype was detected in 17 subjects (22.6%), CT in 33 subjects (44.0%), and TT in 25 subjects (33.3%). In Group III, CC genotype was found in 50 subjects (66.6%), CT in 19 subjects (25.3%), and TT in 6 subjects (8.0%). Among the first-degree relatives of patients (Group II), CC was found in 14 subjects (18.6%), CT in 32 subjects (42.6%), and TT in 29 subjects (38.6%). The TT and CT genotypes were found to be significantly more frequent in Group I and II (Table III).

The presence of the TT genotype was found to increase the frequency of AAA independently in multivariate logistic regression analysis containing all patient and control populations (OR 12.25, 95% CI: 4.300-34.923, $p<0.001$) (Table IV). The presence of CT genotype was also found to increase the frequency of AAA independently (OR 5.108, 95% CI: 2.232-11.236, $p<0.001$).

Table I: Clinical and demographic characteristics of the subjects.

Variable	Group I (n=75)	Group II (n=75)	Group III (n=75)	*p* value
Age (years)	65.34±7.29	50.45±15.67	56.33±14.35	<0.001
Male (n,%)	53 (70.6%)	49 (65.3%)	48 (64.0%)	<0.001
Smoker (n,%)	61 (81.3%)	58 (77.3%)	63 (84.0%)	<0.001
Hypertension (n,%)	64 (85.3%)	59 (78.6%)	57 (76.0%)	<0.001
Hyperlipidemia (n,%)	66 (88.0%)	69 (92.0%)	31 (41.3%)	<0.001
Elevated CRP	67 (89.3%)	59 (78.6%)	17 (23.3%)	<0.001
Diabetes Mellitus (n,%)	14 (18.6%)	10 (13.3%)	13 (17.3%)	<0.001
CAD (n,%)	21 (28.0%)	18 (24.4%)	20 (26.6%)	<0.001
COPD (n,%)	13 (17.3%)	9 (12.0%)	10 (13.3%)	<0.001

CRP: C-reactive protein, **CAD:** Coronary artery disease, **COPD:** Chronic obstructive pulmonary disease, **Group I:** Patient group, **Group II:** First-degree relatives of the patients, **Group III:** Control group.

Table II: Mean aortic diameters of the subject groups.

	Group I n=75	Group II n=75	Group III n=75	*p* value
Diameter (mm)	53.2334*	24.8767	25.9765	<0.001

Group I: Patient group, **Group II:** First-degree relatives of the patients, **Group III:** Control group.
***:** In a post-hoc analysis, aortic diameter of patients in Group I were significantly higher than Group II and Group III.

Table III: Distribution of the MDR1:c.3435C>T polymorphism in patients, relatives of the patients, and the control group.

Genotype	Group I n=75	Group II n=75	Group III n=75	*p* value
CC (Wild Type)	17 (22.6%)	14 (18.6%)	50 (66.6%)*	<0.001
CT (Heterozygous)	33 (44%)**	32 (42.6%)**	19 (25.3%)	<0.001
TT (Homozygous)	25 (33.3%)**	29 (38.6%)**	6 (8.0%)	<0.001

Group I: Patient group, **Group II:** First-degree relatives of the patients, **Group III:** Control group.
***** $p<0.001$ in a post-hoc analysis, comparing group III with group I and group II.
****** $p< 0.001$ in a post-hoc analysis, comparing group I and group II with group III.

Table IV: Independent predictive value of presence of *MDR1* gene mutation in the development of AAA in all groups (n=225).

	OR	95% Confidence interval	*p* value
TT (Homozygous)	12.25	(4.300-34.923)	<0.001
CT (Heterozygous)	5.108	(2.232-11.236)	<0.001

DISCUSSION

We aimed to investigate the association between MDR1 gene and abdominal aortic aneurysm (AAA). Previous studies have investigated the genetic influence on AAA; the hereditary aspect of AAA was first reported by Clifton in 1977 (7-9). As the effect of genetic factors is high, there are many genetic theories aimed at explaining the higher incidence of AAA in male members of the affected families (7,10-18). Shteinberg et al. have shown that the inflammatory response, along with genetic predisposition, is an important risk factor in AAA (19). This was tried to be explained by studies on genes effecting the chemokine receptors with important role in the inflammatory process and on gene deletions that play a role in oxidative stress such as endothelial nitric oxide synthase (eNOS) and by many other pathogenetic studies (7). There are also more recent studies on the roles of inflammation and proteolytic process in the pathophysiology and epidemiology of AAA (21-23). AAA was found to be ten times more frequent in the first-degree relatives of patients with AAA. For this reason, we screened for the *MDR1* gene polymorphism in the first-degree relatives of patients with AAA. The *MDR1* CC (Wild Type), CT (Heterozygous), and TT (Homozygous) genotypes in Group I (who have AAA) and Group III (who have no AAA) were compared. In Group I, the CC genotype was found in 17 subjects (22.6%), the CT genotype in 33 subjects (44.0%), and the TT genotype in 25 subjects (33.3%). In Group III, the CC genotype was found in 50 subjects (66.6%), the CT in 19 subjects (25.3%), and the TT in 6 subjects (8.0%). In Group II, the CC genotype was found in 14 subjects (18.6%), the CT in 32 subjects (42.6%), and the TT in 29 subjects (38.6%). The TT and CT genotypes were found to be significantly more frequent in Group I and Group II ($p<0.001$) than in Group III (Table 3). This finding clearly shows that patients with AAA and their first-degree relatives carry similar characteristics in terms of the 3435C>T polymorphism in the *MDR1* gene.

Having found a significantly higher polymorphism in the *MDR1* gene in patients with AAA and their first-degree relatives, the mechanisms through which the *MDR1* gene may have influence over AAA were evaluated in light of the literature. In previous studies, an increased level of LDL was found in patients with AAA; increased LDL levels were suggested to cause matrix degeneration by inflammation and thus predispose to aneurysm. In a study including 206 AAA patients and 252 healthy individuals, Hobbs *et al.* has found that the LDL levels were significantly higher in the former group (14). In the present study, 66 subjects in Group I (88.0%) had hyperlipidemia compared with 69 subjects in Group II (92.0%) and 31 subjects in Group III (41.3%).

The presence of a similar lipid profile in Group I and Group II, which may be due to genetic or socio-economic similarities between the two, suggests that the relatives of the patients with AAA, which is a disease ascribed to a multitude of factors, may be at a higher risk for developing AAA and should also be monitored.

Although the genetic basis is not clearly known, it was claimed in studies where C-reactive protein (CRP) levels were found to be elevated in the AAA patients that this protein might be a marker of progression. P-glycoprotein (P-gp) was shown to be involved in the transmembrane transport of pro-inflammatory cytokines (20). In experimental studies, a decrease in P-gp secretion and activity was observed in acute inflammation (20). Similarly, P-gp expression was decreased in *MDR1* mutation (21). In our study, the mean CRP level was 71 ±16.2 in patients with AAA. We believe that the CRP levels may be important not in diagnosis of aneurysm, but in monitoring its progression.

There are human and animal studies that suggest a role for matrix metalloproteinases (MMP) in the development of AAA along with serine and cysteine proteases, which are the main proteolytic enzymes. Transmural inflammation was observed in histological materials obtained from aneurysm tissues, which was suggested to be through the contributions of leukocyte-free MMP products produced and activated directly or by cytokine-mediation. MMP-2 and MMP-9 are two mediators with demonstrated effects in the development of AAA through their effects on cell renewal and on macrophages. In a study on cases with drug resistant breast cancer, it was suggested that the P-gp transport protein, which is a product of *MDR1* gene, might be important in the expression of MMP-2 and MMP-9; a significant increase was found in MMP-2 and MMP-9 levels of these cases when they were treated with P-gp substrate (18). This suggests that the polymorphism in *MDR1* gene may have an effect in AAA development through this mechanism. Also, there are studies suggesting a significant effect of MMP-9 on slow or aggressive progression of AAA (24). In addition, other studies have found an increase in MMP-3 expression as a mild risk factor for the development of AAA (25).

CONCLUSION

In line with the results, we suggest a close monitoring of lipid profiles of patients with AAA carrying a polymorphism in *MDR1* gene and their relatives since inflammation and lipid profiles are associated with this gene and other factors that play a role in the etiology of the this disorder. We think that it may be more beneficial to prefer an option with anti-inflammatory effects when choosing a lipid-lowering drug for the individuals at risk due to high levels of LDL.

Finally, the scope of this study and genetic results include a short period of observation since the main aim of this study was to investigate the association between the polymorphism in *MDR1* gene and AAA. For this reason, long-term follow-up of the relatives of patients with AAA was not done. However, the findings of this study related to both genetic and serologic similarities between patients and their relatives suggest that more comprehensive and extended studies monitoring both the risk factors and aortic diameters may be more appropriate in order to more clearly determine the risk ratios of these groups. The present study may provide a base for the future studies.

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