

## Associations between common 3435 C>T variants of the multi-drug resistance [MDR-1 (ABCB1)] gene and abdominal aortic aneurysm: a pilot study

Multi-drug resistance (MDR-1 (ABCB1)) 3435 geni C>T varyantı ve abdominal aort anevrizması arasındaki ilişki: Pilot çalışma

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**Background:** The aim of the study was to reveal the effect of the C3435T MDR-1 gene polymorphism in AAA, which has been postulated to play a role in the inflammatory process and protection against oxidative stress.

**Methods:** In this study, we scanned the MDR gene polymorphisms in peripheral blood samples of the 58 patients (41 males, 17 females; mean age 62.9±6.6 years) whom were operated on after the diagnosis of AAA, and of the 58 healthy individuals (38 males, 20 females; mean age 58.8±11.6 years) have normally measured aorta diameters on abdominal computed tomography scan.

**Results:** We found that MDR-1 C3435T gene CT variant ( $\chi^2=5.80$ ;  $p=0.016$ ) and MDR-1 C3435T gene TT variant ( $\chi^2=11.47$ ;  $p=0.001$ ) polymorphisms was statistically significant in AAA cases ( $p<0.05$ ). The demographic findings were similar in each group.

**Conclusion:** These obtained preliminary results suggest that the T allele polymorphism of the MDR-1 gene is associated with AAA. We believe that such molecular studies will blaze a trail for future studies on the understanding of AAA etiology.

**Key words:** Abdominal aortic aneurysm; inflammation; MDR-1 gene polymorphism; reverse hybridization.

The abdominal aortic aneurysm (AAA) is a localized degenerative disease with a rate of 9% in elderly individuals over 65-years-old. The male gender, smoking, atherosclerosis, high blood pressure, genetic factors and alterations in constitutions of elastin and collagen are considered to be responsible for the etiology.<sup>[1-3]</sup>

**Amaç:** Bu çalışmada, inflamatuvar süreç ve oksidatif strese karşı rol oynadığı ileri sürülen C3435T multi drug rezistans-1 (MDR-1) gen polimorfizminin AAA'daki etkisi ortaya konuldu.

**Çalışma planı:** Bu çalışmada servisimizde AAA tanısı konulduktan sonra ameliyat edilen 58 hasta (41 erkek, 17 kadın; ort. yaş 62.9±6.6) ile abdominal bilgisayarlı tomografi taramasında aort çapları normal olarak ölçülen 58 sağlıklı kişinin (38 erkek, 20 kadın; ort. yaş 58.8±11.6 yıl) periferik kan örneklerinde MDR gen mutasyonu tarandı.

**Bulgular:** MDR-1 C3435T geni CT varyant ( $\chi^2=5.80$ ,  $p=0.016$ ) ve MDR-1 C3435T geni TT varyant ( $\chi^2=11.47$   $p=0.001$ ) polimorfizmleri AAA'lı hastalarda istatistiksel olarak anlamlı bulundu ( $p<0.05$ ). Demografik bulgular gruplar arasında benzerdi.

**Sonuç:** Elde edilen ilk sonuçlar MDR-1 geni T alleli polimorfizminin AAA ile ilişkili olduğunu düşündürmektedir. Bu ve benzeri moleküler çalışmaların AAA etyolojisinin anlaşılmasında gelecekte yapılacak çalışmalara öncü olacağı kanaatindeyiz.

**Anahtar sözcükler:** Abdominal aort anevrizması; inflamasyon; MDR-1 gen polimorfizmi; revers hibridizasyon.

Although the disease is associated with aging, atherosclerosis and familial tendency, its pathophysiology has not been revealed accurately.<sup>[1]</sup>

The abdominal aortic aneurysm enlarges over time and if it has not been controlled, the final result is rupture.<sup>[4]</sup> The incidence of the disease is gradually

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increasing.<sup>[5]</sup> Approximately 50% of AAA will result in rupture if left untreated. Open surgery and endovascular aneurysm repair (EVAR) have been shown to be effective in preventing rupture. Even after surgical treatment, the mortality rate is about 15-30%, but there is lower morbidity risk with EVAR.<sup>[6]</sup> Long-term follow-up is required.<sup>[5]</sup> Furthermore, a second approach may be required in treatment with EVAR.<sup>[6]</sup>

The evaluation of molecular mechanisms that can lead to this pathology will be beneficial to explaining the pathophysiology of the disease.

The genetic transmission was first determined by Clifton in 1977.<sup>[7]</sup> The autoimmune diseases with genetic transmission were also described as a risk factor for AAA.<sup>[8]</sup>

Multi-drug resistance (MDR) gene is a gene group which is considered to be responsible for drug resistance. The gene of MDR-1 causes expression of the P-glycoprotein (P-gp), which takes place in active transport of various substrates. This gene play a role in elimination of toxic substances, intake of nutrients, transport of ions and peptides, and cellular signal transduction.<sup>[9]</sup> The expression of P-gp, a transport protein dependent on ATP, is decreased when there is a single nucleotide polymorphism in 26<sup>th</sup> exon of the MDR-1 gene.<sup>[10-12]</sup>

Furthermore, the evidence of previous studies has shown that cholesterol ester level binding to MDR-1 gene expression is positively correlated with cellular proliferation.<sup>[10]</sup> Besides it has been shown that MDR-1 was observed in pathologies that affect the vessel wall such as atherosclerosis.<sup>[10]</sup> In this study we aimed to investigate the relationship between MDR-1 gene polymorphism and AAA.

## PATIENTS AND METHODS

### Subjects

The local ethics committee approved the study protocol and all subjects gave informed consent. Our patient group consisted of 58 AAA patients (41 males, 17 females; mean age 62.9±6.6 years). The abdominal aortic aneurysms were diagnosed by computed tomography (CT)-scan of thorax and abdomen, defined as a focal dilation of the aorta at least 50% larger than the expected normal diameter. Our control group consisted of 58 healthy subjects (38 males, 20 females; mean age 58.8±11.6 years) who were selected randomly from age-matched adult volunteers who had no focal dilatation of the abdominal aorta 50% larger than the expected normal diameter on CT-scans of the thorax and abdomen taken for other clinical reasons.

### Polymorphism analysis

Total genomic DNA was extracted from 10  $\mu$ l blood samples by the Invitex kit extraction technique (Invitex, Invisorb spin blood, Germany). The multidrug transporter P-gp MDR-1 gene from healthy controls and patients with AAA were simultaneously amplified in a biotin-labeled single multiplex amplification reaction (Viennelab, PGX-HIV Strip Assay, Austria) and evaluated for 3435 C>T polymorphism. The polymerase chain reaction (PCR) was performed in a Perkin Elmer 9600 and the protocol consisted of an initial melting step of 2 minutes at 94 °C; followed by 35 cycles of 15 seconds at 94 °C, 30 seconds at 58 °C, and 30 seconds at 72 °C; and a final elongation step of 3 minutes at 72 °C. The polymorphism analysis was performed by Strip Assay technique (Vienna Lab, PGX-HIV Strip Assay GmbH, and Austria), which is based on the reverse-hybridization principle automatically. The normal, heterozygous and homozygous mutant/non-mutant genotype profiles of each of the genes were determined using the enclosed Collector™ sheet for each person.

### Statistical analysis

The data were expressed as mean  $\pm$  standard deviation (SD) or percentage of column total. Analysis was performed by using SPSS for Windows version 13.0 (SPSS Inc, Chicago, IL, USA). Features of the patients such as sex, hypertension, diabetes mellitus, hyperlipidemia, smoking, coronary artery disease, chronic obstructive pulmonary disease, and the genotype frequencies were evaluated with chi square test. Parametric data were compared with t-test for independent samples. The test of importance between the mean of two groups was used for accordance of two groups and margin of error was set at  $p < 0.05$ .

## RESULTS

In the study group there were 33 cases with hypertension (HT), eight cases with hyperlipidemia (HL), seven cases with diabetes mellitus (DM), 10 cases with chronic obstructive pulmonary disease (COPD), 21 cases with coronary artery disease (CAD) and 21 cases with smoking history. In the control group there were 33 individuals with HT, nine with DM, nine with HL, six with COPD, 21 with CAD and 19 smokers.

The mean abdominal aortic diameter was 54.9±8.6 mm in AAA cases and 26.4±2.9 mm in the control group.

Both groups were similar in terms of demographic data. The demographic data of the study and control groups are summarized in table 1.

Multi-drug resistance-1 genotype distribution and allele frequencies in each group are presented in

**Table 1. The demographic datas of control and patients with abdominal aortic aneurysm**

	Control		Patients with AAA		p
	n	%	n	%	
Sex					
Male	38	65.5	41	70.7	0.55
Female	20	34.5	17	29.3	0.55
Hypertension	31	53.4	33	56.9	0.71
Diabetes mellitus	7	12.1	7	12.1	1.0
Hyperlipidemia	9	15.5	8	13.8	0.79
Smoking	19	32.8	21	36.8	0.69
Coronary artery disease	21	36.2	21	36.2	1.0
Chronic obstruktive pulmonary disease	6	10.3	10	17.2	0.21

AAA: Abdominal aortic aneurysm.

tables 2 and 3. In the patient group, TT variant was detected in 20 (34.5%) patients, CT was detected in 24 (41.3%) patients, and CC was detected in 14 (24.2%) patients. In the control group, TT variant was detected in five (8.6%) patients, CT was detected in 12 (20.7%) patients, and CC was detected in 41 (70.7%) patients. MDR-1 homozygote T/T genotype polymorphism was more frequently observed in the patient group than in the control group ( $p=0.001$ ), just as C3435T allele frequency ( $p=0.001$ ). Based on univariate analysis, having T/T polymorphism was associated with 5.8 fold increased risk of having AAA.

## DISCUSSION

The abdominal aortic aneurysm is a degenerative process which commonly results from aging. The intimal and medial degeneration due to various etiological factors, such as adaptive dilatation, wall attenuation, thinning of atheromatous plaque, inflammatory cellular infiltration, proteolysis, thinning of medial layer as a consequence of impairment the arterial nutrition and adventitial thickening are observed. Proteolytic enzyme activation, inflammation, genetic tendency, infection and hemodynamic effects play a role in pathogenesis of the disease.<sup>[13,14]</sup>

Factors such as age, male gender, smoking, CAD, COPD, HT, DM cause adaptive alterations in AAA.<sup>[2,3,15]</sup> In our study, there were 33 individuals with HT, eight with HL, seven with DM, 10 with COPD, 21 with CAD and 21 smokers, while the mean age of patients with AAA was  $62.9\pm 6.6$  years.

Apart from being associated with different drug levels, studies have suggested that MDR polymorphism may be significant in many diseases such as Parkinson disease, inflammatory bowel diseases, refractory epilepsies in cerebral arterial aneurysms, and regeneration of CD4 cells during treatment of HIV.<sup>[5,6,16]</sup> Previous studies have mentioned that MDR-1 protein plays a role in defense mechanisms versus toxic effects of

smoking and may be effective in removal of stress metabolites.<sup>[17,18]</sup> Furthermore, there are studies mentioning its role in cellular regeneration.<sup>[19]</sup> Shteinberg et al.<sup>[20]</sup> shown that inflammatory response is an important risk factor in AAA, together with genetic predisposition. The proinflammatory cytokines have been shown in the content of P-gp in the cellular secretion.<sup>[21]</sup> In experimental studies, a reduction has been observed in secretion and activity of P-gp in acute inflammation.<sup>[22]</sup> Accordingly, expression of P-gp decreases also in MDR-1 polymorphism.<sup>6</sup> On the other hand present reports show that the production of P-gp regulated by MDR increases MMP-2 and MMP-9.<sup>[23]</sup> It is already known that MMP-2 and MMP-9 play a role in enhancing aneurysm formation.<sup>[24]</sup> MDR can play a role in aneurysm formation through these mentioned mechanisms. In our study MDR-1 C3435T gene CT variant ( $\chi^2 \rightarrow 5.80$ ;  $p=0,016$ ) and MDR-1 C3435T gene TT variant ( $\chi^2 \rightarrow 11.47$ ;  $p=0.001$ ) MDR-1 polymorphism were significantly higher ( $p<0.05$ ) in patients with AAA (Table 3). We also detected that T allele frequency for MDR-1 C3435T gene was significantly higher in patients with AAA ( $p<0.05$ ; Table 2). As a result of our study, we detected that homozygote and heterozygote polymorphisms of MDR-1, which take place in serious metabolic activity, may be associated with AAA. Polymorphism of this gene may cause defects in one or more metabolic functions in which it is involved and may cause development of AAA.

**Table 2. 3435 C>T allele frequency of MDR-1 gene in control and patients with abdominal aortic aneurysm**

	Patient with AAA		Control	
	n	%	n	%
Total allele	116		116	
C allele	52	44.8	98	81.1
T allele*	64	55.2	22	18.9

AAA: Abdominal aortic aneurysm.

**Table 3. Genotype distribution and allele frequency for MDR-1 C3435T polymorphism in patient and control groups**

Genotype	Allele frequency	Patient (n=58)		Control (n=58)		p	OR (95% CI)
	%	n	%	n	%		
Cytosine-cytosine		14	24.20	41	70.69	0.000	
Cytosine-thymine		24	41.30	12	20.69	0.016	
Thymine-thymine		20	34.50	5	8.63	0.001	5.58 (1.9-15.59)
C3435T	0.55 ve 0.19					0.000	5.26 (2.92-9.46)

TT: Homozygous carrier of MDR-1 gene C3435T variant; CT: Heterozygous carrier of MDR-1 gene C3435T variant; CC: Homozygous normal; C3435T: Variant allele.

In conclusion, AAA is a disease which is progressive and highly lethal, requiring its prevention because of both high morbidity and labor loss and high treatment costs despite early intervention. Understanding the etiology of the disease will be one of the most important steps in its treatment and prophylaxis. If the genome is revealed which leads to this disease with multifactorial etiology, individuals under threat of AAA can be easily detected and possible problems can be prevented earlier with less cost. We believe that genetic predisposition should be investigated in patients with AAA.

#### Declaration of conflicting interests

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