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Original Article / Özgün Makale

Relationship of genetic factors with development of aortic dissection and aneurysm

Aort anevrizması ve aort diseksiyonu gelişiminin genetik faktörler ile ilişkisi

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ABSTRACT

Background: This study aims to investigate the relationship between the development of aortic dissections and aneurysms with the polymorphisms of angiotensin converting enzyme gene, methylenetetrahydrofolate reductase gene, plasminogen activator inhibitor-1 gene, and nitric oxide synthase gene.

Methods: Between April 2009 and July 2014, 38 patients with aortic dissections (28 males, 10 females; mean age 55.1±10.7 years; range, 30 to 78 years) and 67 patients with aortic aneurysms (57 males, 10 females; mean age 63.0±11.4 years; range, 31 to 82 years) were included in this cross-sectional study. The control group consisted of 60 healthy volunteers (41 males, 19 females; mean age 56.3±11.2 years; range, 30 to 82 years) without an aortic aneurysm or dissection, as assessed by thoracoabdominal computed tomography. The prespecified four genes were genotyped with competitive allele-specific polymerase chain reaction.

Results: The aortic dissection group had higher nitric oxide synthase-3 (4b/4b) expression levels, compared to the control group. The aortic aneurysm group had also higher nitric oxide synthase-3 (4b/4a) expression levels, compared to the control group. Compared to the control group, a higher rate of angiotensin converting enzyme I/D gene polymorphism was detected in the aneurysm group, while higher D/D polymorphism rates were found in the dissection group; although not statistically significant.

Conclusion: Our study results suggest that the nitric oxide synthase-3 intron 4b/4b and nitric oxide synthase-3 intron 4b/4a gene polymorphisms can be used as a predictor of aortic dissection and aneurysm development.

Keywords: Aortic aneurysm; aortic dissection; genetic; nitric oxide synthase-3; NOS3; polymorphism.

ÖZ

Amaç: Bu çalışmada aort diseksiyonu ve anevrizma gelişimi ile anjiyotensin dönüştürücü enzim geni, metilentetrahidrofolat redüktaz geni, plazminojen aktivatör inhibitör-1 geni ve nitrik oksit sentaz geni polimorfizmleri arasındaki ilişki araştırıldı.

Çalışma planı: Bu kesitsel çalışmaya Nisan 2009 - Temmuz 2014 tarihleri arasında aort diseksiyonu olan 38 hasta (28 erkek, 10 kadın; ort. yaş 55.1±10.7 yıl; dağılım 30-78 yıl) ve aort anevrizması olan 67 hasta (57 erkek,10 kadın; ort. yaş 63.0±11.4 yıl; dağılım 31-82 yıl) alındı. Kontrol grubu, torakoabdominal bilgisayarlı tomografi ile değerlendirildiği üzere, aort anevrizması veya diseksiyonu olmayan 60 sağlıklı gönüllüden (41 erkek, 19 kadın; ort. yaş 56.3±11.2 yıl; dağılım 30-82 yıl) oluşuyordu. Belirtilen dört genin, kompetitif allel spesifik-polimeraz zincir reaksiyonu ile genotiplemesi yapıldı.

Bulgular: Kontrol grubuna kıyasla, aort diseksiyonu grubunda nitrik oksit sentaz-3 (4b/4b) ekspresyon düzeyi daha yüksekti. Kontrol grubuna kıyasla, aort anevrizması grubunda nitrik oksit sentaz-3 (4b/4a) ekspresyon düzeyi daha yüksekti. İstatistiksel olarak anlamlı olmamakla birlikte, kontrol grubuna kıyasla, anevrizma grubunda daha yüksek oranda anjiyotensin dönüştürücü enzim I/D gen polimorfizmi saptanır iken, diseksiyon grubunda daha yüksek D/D polimorfizm oranlarına rastlandı.

Sonuç: Çalışma sonuçlarımız, nitrik oksit sentaz-3 intron 4b/4b ve nitrik oksit sentaz-3 intron 4b/4a gen polimorfizmlerinin, aort diseksiyonu ve anevrizma gelişiminin bir öngördürücüsü olarak kullanılabileceğini göstermektedir.

Anahtar sözcükler: Aort anevrizması; aort diseksiyonu; genetik; nitrik oksit sentaz-3; NOS3; polimorfizm.

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Aortic dissections and aneurysms are life-threatening conditions associated with high morbidity and mortality. Aortic dissection is an aortic pathology characterized by a tear in the intima layer which separates the media layer from the bloodstream, leading to the development of a false lumen between the intima and media layers of the aorta.[1] Aneurysm is an abnormal and irreversible dilatation (two times greater than normal diameter) which can affect any segment of the aorta. Aortic aneurysms are often caused by degenerations which can be also related with dissections, connective tissue diseases, blunt traumas, aortitis, mycotic infections, or congenital anomalies.[2]

Aortic aneurysms and dissections are important health issues in terms of mortality rates and other health services all over the world.[1] The prevalence of aortic aneurysms is between 1.7 and 12.7%.[2] However, it is difficult to determine the true incidence, as aortic dissections usually remain undiagnosed. The prevalence of aortic dissections is reported to be between 0.2 and 0.8%.[2] In a study where 12-year retrospective data was evaluated, the incidence of both diseases was found to increase over the years. Of note, aortic dissections and aortic aneurysms are more common in men than in women.[3,4]

In the literature, there are several studies examining the relationship between the development of aortic dissections and aneurysms and genetic factors. In the present study, we aimed to investigate the relationship between the development of aortic dissections and aneurysms and the polymorphisms of angiotensin converting enzyme (ACE) gene, methylenetetrahydrofolate reductase (MTHFR) gene, plasminogen activator inhibitor-1 (PAI-1) gene, and nitric oxide synthase (NOS) gene.

PATIENTS AND METHODS

This cross-sectional study included a total of 38 patients with aortic dissections (28 males, 10 females; mean age 55.0±10.7 years; range, 30 to 78 years) and 67 patients with aortic aneurysms (57 males, 10 females; mean age 63.0±11.4 years; range, 31 to 82 years) at Erciyes University, Faculty of Medicine, Department of Cardiovascular Surgery between April 2009 and July 2014. The patients were divided into three groups as the control, aneurysm, and dissection group. Patients with aortic dissections were referred to as the dissection group and patients with aortic aneurysms were referred to as the aneurysm group. The dissection group was further divided into two groups according to the Stanford classification: Stanford type A group (n=33) and Stanford type B group (n=5). Aortic dissections and aneurysms were diagnosed with computed tomography and echocardiography (Figures 1, 2). The patients with a detectable dissection flap and those with an enlarged aortic diameter (two-times greater than normal diameter) in any segment of the aorta in the transverse measurements were included in the study group.

The control group consisted of 60 healthy volunteers (41 males, 19 females; mean age 56.3±11.2 years; range, 30 to 82 years) who did not have an aortic aneurysm or dissection, as assessed by the thoracoabdominal computed tomography and transthoracic echocardiography. Patients with a disease which may cause aneurysms such as Marfan syndrome or Behçet's disease and those with a syndromic phenotype were excluded from the study.

The study protocol was approved by the Erciyes University, Faculty of Medicine, Ethics Committee (E.C. Number: 09/202, Date: 07.04.2009). A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data collection

A 5 mL blood sample was taken with vacutainer from each individual. Blood samples were stored at +4°C in a tube containing 1/100 volume and 0.5 mmol/L sodium ethylenediaminetetraacetic acid (EDTA).

Genetic testing

All of the genetic studies were performed in the Genome and Stem Cell Center at Erciyes

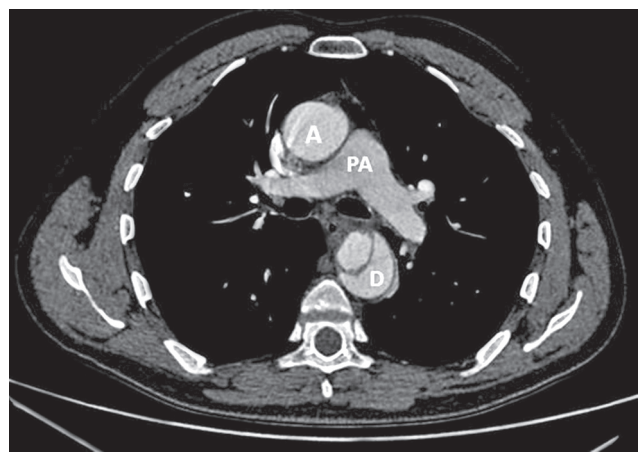

Figure 1. A standard axial image from a computed tomography scan demonstrating a descending thoracic aortic aneurysm and dissection.
A: Ascending aorta; D: Descending aorta; PA: Pulmonary artery.

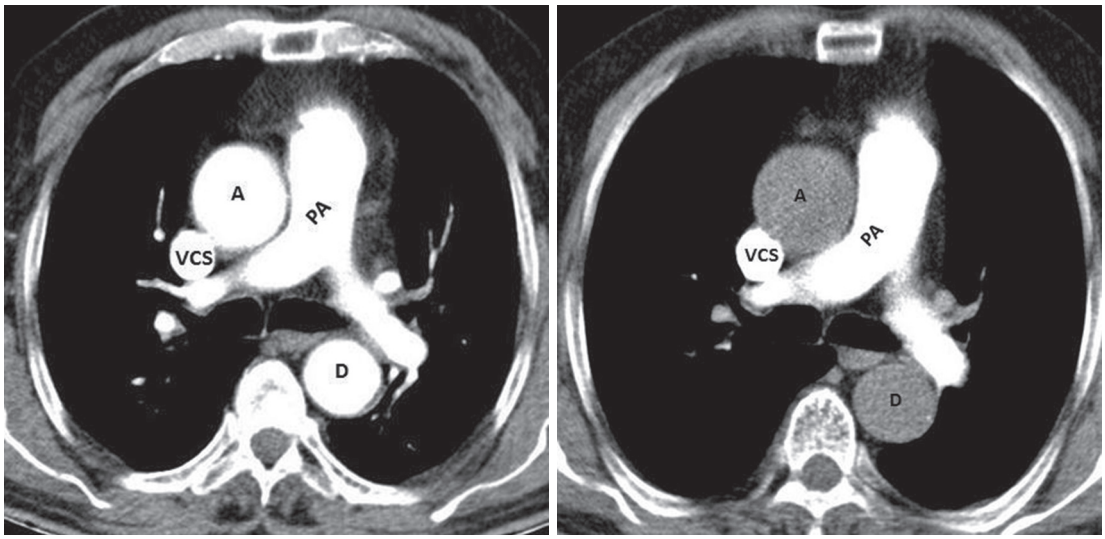

Figure 2. A standard axial image from a computed tomography scan demonstrating an ascending aortic aneurysm.
A: Ascending aorta; D: Descending aorta; PA: Pulmonary artery; VCS: Vena cava superior.

University (GENKOK). A 2 mL of blood samples with EDTA were obtained from each participant. Genomic deoxyribonucleic acid (DNA) was extracted from the peripheral blood samples using standard procedures of High Pure PCR Template Preparation Kit (Roche, Germany). The final DNA concentration was determined with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). Amplifications of NOS3 intron 4 polymorphism (rs61722009) were performed with standard polymerase chain reaction (PCR). The PCR products were visualized with 2% agarose gel stained with ethidium bromide. The NOS3 Glu298Asp (894G>T, rs1799983) polymorphism was determined using a PCR-restriction fragment length polymorphism (RFLP)-based protocol. The PCR products were checked on 2.5% agarose gel, 206-bp eNOS product was digested overnight at 37°C with Mbo I restriction endonuclease enzyme.[3]ACE gene I/D polymorphism were amplified with PCR method. Fifty microliters final volume of PCR mixture consisted of 50 ng genomic DNA, 10×PCR buffer, 0.2 mM of each dNTP, MgCl2 (1.5 mM), Taq DNA polymerase (1 U/mL), and 10 pmol of each primer.[4] The ACE deletion polymorphism is characterized by a 190-bp fragment, whereas the presence of the insertion leads to a 490-bp fragment. PAI-1 4G/5G amplification was performed for 30 cycles

Table 1. Primer sequences and polymerase chain reaction program for genotyping gene polymorphisms

Polymorphisms	Polymerase chain reaction primers	Annealing temperature (°C)
NOS3Intron 4 (4a/4b)	Forward 5'-AGGCCCTATGGTAGTGCCTT-3' Reverse 5'-TCTCTTAGTGCTGTGGTCAC-3'	56
NOS3 894G>T	Forward 5'-CATGAGGCTCAGCCCCAGAAC-3' Reverse 5'-AGTCAATCCCTTTGGTGCTCAC-3'	60
ACE I/D	Forward 5'-CTGGAGACCACTCCCATCCTTTCT-3' Reverse 5'- GATGTGGCCATCACATTCGTCAGAT-3'	57
PAI-1 4G/5G	Forward 5'- CACAGAGAGAGTCTGGCCACGT-3' Reverse 5'- CCAACAGAGGACTCTTGGTCT--3'	60
MTHFR C677T	Forward 5'- TGAAGGAGAAGGTGTCTGCGGGA -3' Reverse 5'- AGGACGGTGCGGTGAGAGTG --3'	62

NOS: Nitric oxide synthase; ACE: Angiotensin converting enzyme; PAI-1: Plasminogen activator inhibitor; MTHFR: Methylenetetrahydrofolate reductase.

Table 2. Genotypes of related polymorphisms

Polymorphism	Restriction enzyme	PCR products	Normal genotype	Heterozygotegenotype	Homozygotegenotype
PAI-1 4G/5G	Bsl I	98 bp	98 bp	98 bp 77 bp 22 bp	77 bp 22 bp
ACE I/D	-	490 bp	490 bp	490 bp 190 bp	190 bp
NOS3 intron 4	-	-	420 bp	420 bp 393 bp	393 bp
NOS3 G894T	Mbo I	206 bp	206 bp	206 bp 119 bp 87 bp	119 bp 87 bp
MTHFR C677T	Hinf I	198 bp	198 bp	198 bp 175 bp 23 bp	175 bp 23 bp

PCR: Polymerase chain reaction; PAI-1: Plasminogen activator inhibitor; ACE: Angiotensin converting enzyme; NOS: Nitric oxide synthase, MTHFR: Methylenetetrahydrofolate reductase.

Table 3. Comparison of groups in terms of genetic variables

	Control group (n=60)			Aneurysm group (n=67)			Dissection group (n=38)			
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (year)			56.3±11.2			63.0±11.4			55.0±10.7	**<0.05**
Gender										>0.05
Male	41			57			28			
Female	19			10			10			
PAI-1 4G\5G										0.590
Normal	13	21.7		23	34.3		11	28.9		
Heterozygous	32	53.3		32	47.8		18	47.4		
Homozygous	15	25.0		12	17.9		9	23.7		
ACE										0.219
I/I	15	25.0		13	19.4		9	23.7		
I/D	27	45.0		34	50.7		11	28.9		
D/D	18	30.0		20	29.9		18	47.4		
NOS3										**0.048**
4b/4b	50	83.3		45	67.2		32	84.2		
4b/4a	10	16.7		22	32.8		6	15.8		
NOS3										0.395
GG	29	48.3		35	52.2		22	57.9		
GT	23	38.3		29	43.3		12	31.6		
TT	8	13.3		3	4.5		4	10.5		
MTHFR C677T										0.948
Normal	24	40.0		31	46.3		16	42.1		
Heterozygous	34	56.7		34	50.7		20	52.6		
Homozygous	2	3.3		2	3.0		2	3.6		

SD: Standard deviation; PAI-1: Plasminogen activator inhibitor; ACE: Angiotensin converting enzyme; NOS: Nitric oxide synthase; MTHFR: Methylenetetrahydrofolate reductase; Bold value indicates a statistically significant difference (p<0.05).

with denaturation temperature of 94°C for three min, 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, the final extension step at 72°C was extended by one min. Amplified 98-bp product was digested overnight with Bsl I at 55°C and subjected to 4% agarose gel electrophoresis.[5] MTHFR C677T polymorphism (rs1801133) was shown with PCR-RFLP. PCR cycling conditions included an initial denaturation at 94°C for two min, followed by 40 cycles of 30 sec at 94°C, 30 sec at 62°C, and 30 sec at 72°C, and a final extension step at 72°C for seven min. A total of 198 base pairs of PCR products were, then, digested overnight at 37°C with Hinf I restriction enzyme and checked with 3% agarose gel electrophoresis.[6] All of the PCR primers of the polymorphisms and annealing temperatures are summarized in Table 1. The genotypes of the related polymorphisms after RFLP are also summarized in Table 2.

Statistical analysis

The statistical analysis was performed using the IBM SPSS for Windows, version 21.0 software (IBM Corp., Armonk, NY, USA). First, the control, aneurysm, and dissection groups were compared in terms of variables. Subsequently, the Stanford type A and Stanford type B dissection groups were compared within themselves. Statistical data were expressed in the number of units (n), percentage (%), mean ± standard deviation (SD) and median (25^{th}-75^{th} percentile). The normal distribution of numerical variables was assessed with the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used to compare the means of more than two groups. The Mann-Whitney U test was used to compare the two groups. The chi-square test was used to compare categorical variables. A p value of <0.05 was considered statistically significant.

RESULTS

The age and gender distribution of the groups is shown in Table 3. In terms of age, the mean age of the aneurysm group was statistically higher than the dissection and control groups (p<0.001). However, there were no statistically significant differences among the groups in terms of gender (p>0.05).

There was a statistically significant difference in the NOS3 intron 4 polymorphism between the

Table 4. Dissection groups according to genetic variables

	Dissection group				
	Stanford type A (n=33)		Stanford type B (n=5)		
	n	%	n	%	*p*
PAI-1 4G\5G					0.567
Normal	9	27.3	2	40	
Heterozygous	15	45.5	3	60	
Homozygous	9	27.3	-	-	
ACE					0.567
I/I	9	27.3	-	-	
I/D	9	27.3	2	40	
D/D	15	45.5	3	60	
NOS3					0.570
4b/4b	27	81.8	5	100	
4b/4a	6	18.2	-	-	
NOS3					0.529
GG	18	54.7	4	80	
GT	11	33.3	1	20	
TT	4	12.1	-	-	
MTHFR C677T					1.000
Normal	14	42.4	2	40	
Heterozygous	17	51.5	3	60	
Homozygous	2	6.1	-	-	

PAI-1: Plasminogen activator inhibitor; ACE: angiotensin converting enzyme; NOS: Nitric oxide synthase; MTHFR: Methylenetetrahydrofolate reductase.

dissection group and the control group ($p<0.05$). In addition, there was a statistically significant difference between the aneurysm group and the control group in terms of NOS34b/4a polymorphism ($p<0.05$). However, we found no statistically significant difference between the control group and the patient groups in terms of other variables ($p>0.05$) (Table 3). Also, there were no statistically significant differences between the Stanford type A and Stanford type B groups ($p>0.05$) (Table 4).

DISCUSSION

Previous studies have shown that aortic aneurysms and dissections have a strong genetic background.[1,2] To date, a number of genetic analyses has been used to understand the underlying mechanisms of the development of aortic aneurysms and dissections.[2,3] However, these studies are mostly related to syndromic disorders.[2] The genetic basis of non-syndromic aortic aneurysms and dissections is much more complex. In several studies in which the patients with aortic aneurysms and dissections were included, various non-syndromic mutations were identified in the genetic tests.[2,3] Still, new investigations are needed to determine which gene mutations cause non-syndromic diseases. In this study, we investigated the relationship between the development of aortic dissections and aneurysms and the ACE I/D gene polymorphism, MTHFRC 677T gene polymorphism, PAI-1 4G/5G gene polymorphism, NOS3 intron 4, and NOS3 G894T gene polymorphism.

Many researchers have suggested that genetic components may play a role in the pathogenesis of aortic diseases.[7,8] Researchers have searched for genes encoding key enzymes that play an important role in aneurysms, dissections, and inflammatory responses, and elastin, elastase, collagen, collagenase, metalloproteinase (MMP-1, 8, 13), tissue inhibitors, PAI-1, interleukins, ACE, MTHFR, NOS3, platelet activating factor, human leucocyte antigens, and inflammatory receptors have been investigated.[7,8]

The PAI-1 gene is located on the long arm of chromosome 7 (q21.3-q22). It is an important regulator of plasma activation in the tissue. A polymorphism within the gene sequence of PAI-1 has changed PAI-1 expression and plasminogen activation.[9] The most frequently investigated PAI-1 genetic variation is the 4G/5G insertion/deletion polymorphism. This polymorphism causes the nucleotide sequence of 4 or 5 guanine (4G or 5G), and the emerging different alleles lead to changes in the expression of PAI-1.[10] In their study, Jones et al.[11] found that polymorphism of the PAI-1 4G/5G gene was not related with the development of aortic aneurysms. Also, Rossaak et al.[9] reported that there was no statistically significant difference between the healthy controls and patients with aortic aneurysms in terms of characteristics of the PAI-1 gene distribution. Similarly, in our study, there was no statistically significant difference between the aneurysm and control groups in terms of the PAI-1 4G/5G gene polymorphism.

Subcutaneous angiotensin II infusion in mice has been shown to affect the renal-angiotensin system, which leads to aortic aneurysms. However, previous studies have not elucidated whether ACE gene polymorphism is a risk factor for aortic aneurysms. In a study, Fatini et al.[12] reported that ACE D/D polymorphism was an independent risk factor for the development of aortic aneurysms. On the other hand, Hamano et al.[13] found exact opposite results. In a study where the long-term effects of the ACE I/D genotype on aortic aneurysms were examined, Yeung et al.[14] also reported no association between the ACE I/D and aortic aneurysms. Additionally, Korcz et al.[15] found that ACE I/D gene polymorphism was not a predisposing factor for the development of aortic aneurysms. Consistent with the previous findings, we also showed that the ACE gene polymorphism did not play a role in the development of aortic aneurysms.

In the literature, there is a limited number of data about genetic causes of the aortic dissections. Kalay et al.[16] found that the frequency of D allele in the ACE gene polymorphism significantly increased in aortic dissections.[16] In our study, there was no difference between the aortic dissection and control group. In addition, there was no difference in the development of aortic dissection between the D/D allele and the I/D allele.

It is well-known that nitric oxide (NO), which is regularly released from the endothelium and synthesized by the NOS3 enzyme, has a vasculoprotective effect by providing a regular vasodilatation.[3] Irregular release of the NO leads to aneurysm development, causing weakness in the vessel wall and subsequent injury.[19-21] In addition, irregular NO synthesis is associated with weakened vessel walls by altering the amount of elastin protein, which is an important component of the extracellular matrix.[17]

The clinical and prognostic importance of NOS 4a/b polymorphism has been shown previously in different clinical conditions such as thrombosis, atherosclerosis, and myocardial infarction.[3,4,22] Therefore, negative

effects of nos gene polymorphism may be a risk factor for aneurysm and dissection development. Johanning et al.[18] reported that experimentally-induced aneurysms in rats were inhibited by the administration of NO inhibitors. The gene encoding NOS3 has been proposed as a predisposing gene for aortic aneurysms, and polymorphic variants of this gene affect the release and functional activity of the enzyme. Veldman et al.[19] found that Glu298Asp (G894T) polymorphism in the exon 7 region of the NOS3 gene was associated with decreased basal NO synthesis. Fatini et al.[20] reported that the NOS3 Glu298Asp (G894T) polymorphism predisposed to aortic aneurysms. On the contrary, Moon et al.[21] suggested that there was no association between the Glu298Asp (G894T) polymorphism and plasma NO metabolites. Our study findings are consistent with the findings of the Moon's study. There was no difference between the patients and healthy controls in terms of the NOS3 (G894T) polymorphism.

To the best of our knowledge, there are no studies which investigate the development of aneurysms and dissections in terms of NOS3 intron 4 polymorphism in the literature. Hence, this is the first study on this subject. In this study, NOS3 4b/4b polymorphism in the dissection group and NOS3 4b/4a polymorphism group in the aneurysm group were found to be statistically significantly higher than the control group. These findings indicate that there is a relationship between the development of aortic aneurysms/dissections and NOS3 intron 4 gene polymorphism.

Furthermore, polymorphic variants of the MTHFR gene have been found to be associated with hyperhomocysteinemia, vascular pathologies, neural tube defects, dementia, perinatal mortality, mental disorders, neurodegenerative disorders, migraine, and cancer.[22] Frosst et al.[23] reported an increased risk of aortic aneurysms in MTHFR 677T allele carriers. LaMorte et al.[24] also reported that the MTHFR gene polymorphism was highly correlated with aortic aneurysms in Caucasian males. However, in our study, we found no statistically significant difference between the control and patient groups. It can be attributed to the fact that the ethnicity is different from the reported cohorts. In a previous study, it was reported that individuals with T allele in the Turkish population are fewer than the other races.[25]

The limitations of our study are; it is a single centered study and the number of patients are relatively low.

In conclusion, our study results showed that there was an association between the NOS3 (4b/b, 4b/a) and aortic aneurysms or dissections. Nonetheless, further studies on genetic factors which play an important role in underlying mechanisms of the development of aortic aneurysms and dissections are needed.

Declaration of conflicting interests

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REFERENCES

1. Xenos M, Labropoulos N, Rambhia S, Alemu Y, Einav S, Tassiopoulos A, et al. Progression of abdominal aortic aneurysm towards rupture: refining clinical risk assessment using a fully coupled fluid-structure interaction method. Ann Biomed Eng 2015;43:139-53.
2. Auer J, Berent R, Eber B. Aortic dissection: incidence, natural history and impact of surgery. J Clin Basic Cardiol 2000;3:151-4.
3. Sener EF, Emirogullari ON, Serhatlioglu F, Ozkul Y. The role of endothelial nitric oxide synthase gene G894T and intron 4 VNTR polymorphisms in hemodialysis patients with vascular access thrombosis. Anadolu Kardiyol Derg 2014;14:239-43.
4. Kocyigit I, Taheri S, Sener EF, Unal A, Eroglu E, Öztürk F, et al. Endothelial nitric oxide synthase gene expression is associated with hypertension in autosomal dominant polycystic kidney disease. Cardiorenal Med 2014;4:269-79.
5. Dundar M, Kiraz A, Balta B, Emirogullari EF, Zararsiz G, Yurci A, et al. The role of TNF-α and PAI-1 gene polymorphisms in familial Mediterranean fever. Mod Rheumatol 2013;23:140-5.
6. Sener EF, Oztop DB, Ozkul Y. MTHFR Gene C677T Polymorphism in Autism Spectrum Disorders. Genet Res Int 2014;2014:698574.
7. Jones GT, Phillips VL, Harris EL, Rossaak JI, van Rij AM. Functional matrix metalloproteinase-9 polymorphism (C-1562T) associated with abdominal aortic aneurysm. J Vasc Surg 2003;38:1363-7.
8. Ogata T, Shibamura H, Tromp G, Sinha M, Goddard KA, Sakalihasan N, et al. Genetic analysis of polymorphisms in biologically relevant candidate genes in patients with abdominal aortic aneurysms. J Vasc Surg 2005;41:1036-42.
9. Rossaak JI, Van Rij AM, Jones GT, Harris EL. Association of the 4G/5G polymorphism in the promoter region of plasminogen activator inhibitor-1 with abdominal aortic aneurysms. J Vasc Surg 2000;31:1026-32.
10. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. J Biol Chem 1993;268:10739-45.
11. Jones K, Powell J, Brown L, Greenhalgh R, Jormsjö S, Eriksson P. The influence of 4G/5G polymorphism in the

plasminogen activator inhibitor-1 gene promoter on the incidence, growth and operative risk of abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 2002;23:421-5.

12. Fatini C, Pratesi G, Sofi F, Gensini F, Sticchi E, Lari B, et al. ACE DD genotype: a predisposing factor for abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 2005;29:227-32.
13. Hamano K, Ohishi M, Ueda M, Fujioka K, Katoh T, Zempo N, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is not a risk factor predisposing to abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 1999;18:158-61.
14. Yeung JM, Heeley M, Gray S, Lingam MK, Manning G, Nash JR, et al. Does the angiotensin-converting enzyme (ACE) gene polymorphism affect rate of abdominal aortic aneurysm expansion? Eur J Vasc Endovasc Surg 2002;24:69-71.
15. Korcz A, Mikołajczyk-Stecyna J, Gabriel M, Zowczak-Drabarczyk M, Pawlaczyk K, Kalafirov M, et al. Angiotensin-converting enzyme (ACE, I/D) gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease. J Surg Res 2009;153:76-82.
16. Kalay N, Caglayan O, Akkaya H, Ozdogru I, Dogan A, Inanc MT, et al. The deletion polymorphism of the angiotensin-converting enzyme gene is associated with acute aortic dissection. Tohoku J Exp Med 2009;219:33-7.
17. Kim JU, Chang HK, Lee SS, Kim JW, Kim KT, Lee SW, et al. Endothelial nitric oxide synthase gene polymorphisms in Behçet's disease and rheumatic diseases with vasculitis. Ann Rheum Dis 2003;62:1083-7.
18. Johanning JM, Armstrong PJ, Franklin DP, Han DC, Carey DJ, Elmore JR. Nitric oxide in experimental aneurysm formation: early events and consequences of nitric oxide inhibition. Ann Vasc Surg 2002;16:65-72.
19. Veldman BA, Spiering W, Doevendans PA, Vervoort G, Kroon AA, de Leeuw PW, et al. The Glu298Asp polymorphism of the NOS 3 gene as a determinant of the baseline production of nitric oxide. J Hypertens 2002;20:2023-7.
20. Fatini C, Sofi F, Sticchi E, Bolli P, Sestini I, Falciani M, et al. eNOS G894T polymorphism as a mild predisposing factor for abdominal aortic aneurysm. J Vasc Surg 2005;42:415-9.
21. Moon J, Yoon S, Kim E, Shin C, Jo SA, Jo I. Lack of evidence for contribution of Glu298Asp (G894T) polymorphism of endothelial nitric oxide synthase gene to plasma nitric oxide levels. Thromb Res 2002;107:129-34.
22. Ilhan N, Kucuksu M, Kaman D, Ilhan N, Ozbay Y. The 677 C/T MTHFR polymorphism is associated with essential hypertension, coronary artery disease, and higher homocysteine levels. Arch Med Res 2008;39:125-30.
23. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111-3.
24. LaMorte WW, Scott TE, Menzoian JO. Racial differences in the incidence of femoral bypass and abdominal aortic aneurysmectomy in Massachusetts: relationship to cardiovascular risk factors. J Vasc Surg 1995;21:422-31.
25. Yilmaz H, Isbir S, Agachan B, Ergen A, Farsak B, Isbir T. C677T mutation of methylenetetrahydrofolate reductase gene and serum homocysteine levels in Turkish patients with coronary artery disease. Cell Biochem Funct 2006;24:87-90.