Research Article

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***FBN-1*, *FN-1* and *TIMP-3* gene expression levels in patients with thoracic aortic aneurysm**

Torasik Aort Anevrizmalı Hastalarda *FBN-1*, *FN-1* ve *TIMP-3* Gen Ekspresyon Seviyeleri

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Abstract

Background: Aortic aneurysm occurs in the thoracic and abdominal sections of the aorta and is a deadly late-age-at-onset disease. Thoracic aortic aneurysms (TTAs) are characterized by progressive smooth muscle cell rarefaction due to impaired extracellular matrix. The aim of this study was to investigate fibrillin-1 (FBN-1), fibronectin-1 (FN-1) and tissue inhibitors of metalloproteinases-3 (TIMP-3) gene expression levels in patients with TTA.

Materials and methods: The data were analyzed for 16 patients treated for TAA and nine control subjects. Tissue samples obtained during surgery were frozen immediately in liquid nitrogen and stored at -80°C until RNA isolation. Gene expression analysis was performed by quantitative reverse transcription polymerase chain reaction for each gene and Beta actin was used as control gene. $2^{-\Delta\Delta\text{CT}}$ method was used for the determining expression levels of the genes.

Results: According to the results of this study, TIMP-3 gene was nine-fold higher expressed in TAA tissues ($p = 0.034$). Furthermore, TIMP-3 expression levels were found

associated with fasting blood glucose, red blood cells and ejection fraction. The gene expression levels of FBN-1 and FN-1 were not statistically significant ($p > 0.05$).

Conclusion: In this clinical trial, we concluded that TIMP-3 expression increases in dilated aorta.

Keywords: thoracic aortic aneurysms; TIMP-3; FBN-1; FN-1.

Öz

Amaç: Aort anevrizması aortun torasik ve abdominal bölümlerinde meydana gelir ve ölümcül, bir ileri yaş atak hastalığıdır. Torasik Aort Anevrizma (TAA) ilerleyen düz kas hücresi azalmasına bağlı olarak ekstrasellüler matriksin yıkımı ile karakterize edilir. Bu çalışmada Torasik Aort Anevrizmalı hastalarda fibrillin-1 (FBN-1), fibronektin-1 (FN-1) ve metalloproteinaz doku inhibitörü-3 (TIMP-3) gen ekspresyon seviyelerinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmamıza 16 TAA'lı olgu örneği ve 9 kontrol örneği dâhil edildi. Ameliyat sırasında alınan doku örnekleri sıvı nitrojen içerisinde dondurularak RNA izolasyonu yapılarak -80°C derin dondurucuda saklandı. Kontrol geni olarak beta aktin kullanıldı. Gen ekspresyon analizi qRT-PCR metodu kullanılarak yapıldı. $2^{-\Delta\Delta\text{CT}}$ metodu gen ekspresyon seviyelerinin belirlenmesinde kullanıldı.

Bulgular: TIMP-3 geninin TAA dokularında 9 kat fazla eksprese edildiği bulundu ($p = 0,034$). Dahası, TIMP-3 ekspresyon seviyeleri açlık kan şekeri, kırmızı kan hücreleri ve ejeksiyon fraksiyonuyla ilişkili bulundu. FBN-1 ve FN-1 gen ekspresyon seviyeleri istatistiksel olarak anlamlı bulunmadı ($p > 0,05$).

Sonuç: Çalışmamızda genişlemiş olan aortta TIMP3 ekspresyonunun arttığı belirlenmiştir.

Anahtar kelimeler: Torasik aort anevrizması; TIMP-3; FBN-1; FN-1.

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Introduction

Aortic aneurysms are characterized by abnormal aortic enlargement resulting from a large scale or limited weakening of the vessel wall. This formation is derived from defects in the synthesis of extracellular matrix (ECM) proteins or the accelerated degradation of the vascular ECM [1, 2]. Estimated mortality rates approaching 1% per hour in the first 48 h, reaches up to 80% in the first month [3]. Aortic aneurysms usually result in death [4] and the incidence of acute thoracic aortic dissection is reported as ~2.53/100,000/year [5]. The 1-year survival rate is between 96% and 97.6% and the 3-year survival rate is between 88.3% and 90.5% when surgical intervention is performed rapidly [6]. The ascending thoracic aortic aneurysm (TTA) is associated with various syndromic conditions such as hypertension, bicuspid aortic valve (BAV) and Marfan syndrome (MFS) and causes TAA dissection leading to early life stage mortality [7].

Fibrillin-1 (FBN-1), encodes FBN-1, is the major protein in the microfibrils that connect elastic fibers to dense plaques in smooth muscle cells (SMC) [8, 9]. Altered or diminished FBN-1 can contribute to premature mechanical fatigue or elastolysis which may cause to aneurysm formation [10, 11] and altered mechanosensing [12], FBN-1 plays as sequester latent transforming growth factor β (TGF β) in the ECM, though. It has been suggested that mutations in FBN-1 can lead to ineligible release of active TGF β from the matrix and cause the increased SMAD and mitogen-activating protein kinase (MAPK) signaling in aortas of Marfan mouse models [13, 14]. It has been proposed that the presence of a fibronectin (FN) network is essential for the assembly of FBN-1 in cultures of human dermal fibroblasts [9]. The exact functions of FN-extracellular domain A (EDA) and extracellular domain B (EDB) in the vessel wall are not yet known, however many activities have been attributed to these domains. Studies on mice showed that EDA-containing FN is effective in pulmonary fibrosis formation and wound healing in the skin [15]. Thus, FN containing EDA is believed to play a fundamental role in cell migration, division, tissue repair and maintenance of tissue content [15]. BAV is the most frequently recognized congenital anomaly among adults, occurring in 1–2% of the general population [16, 17]. BAV and TAAs are independent occurrences of a single genetic defect [18]. It was determined that reduced EDA expression causes susceptibility to TAA and FN protein expression levels increase in the presence of aortic aneurysm in BAV patients. Furthermore, it is thought that FN-EDA and FN-EDB play a critical role in vascular morphogenesis [15].

Matrix metalloproteinases (MMPs) impair plaque instability and predispose to vascular complications. Thus, impairing of MMP or tissue inhibitors of metalloproteinases (TIMP) expression is associated with a higher risk of mortality [19]. TIMPs play an important role as the endocrine regulator proteins of MMPs, determine the interaction of the ECM, the cell adhesion molecules and a large number of cytokines [20]. Up to now, four types of TIMP genes have been identified, including TIMP-1, TIMP-2, metalloproteinases-3 (TIMP-3) and TIMP-4 [21]. TIMP-3 was found highly expressed in enlarged aortic tissues compared to control tissues, even though there is no upregulation or down regulation of a particular MMP [22]. TIMP-3 has a structure that interacts interactively with the components of the ECM. If TIMP-3 is overexpressed in human hemopoietic cells, fatal osteosclerosis is unavoidable [23].

Differentially expressed genes were studied in ascending TTAs and several gene expressions were identified. Sulkava et al. [24] concluded that homoeobox genes are potentially important for the maintenance of aortic integrity and smooth muscle, on the other hand alpha-2 actin gene mutation was determined in another study [25]. Although different types of mutations were identified in the pathogenesis of TTAs fibronectin-1 (FN-1), FBN-1 and TIMP-3 genes were not studied before. Thus, the aim of this study was to investigate FBN-1, FN-1 and TIMP-3 gene expression levels in patients with TTA in order to investigate the possible relationship.

Materials and methods

Patient selection

All participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration. The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors. Ethical approval was obtained by the Ethical Committee of our university (2012/435-993). Twenty patients diagnosed with TTAs using aortography, magnetic resonance imaging and computed tomography were included in the study as study group. During the study, four patients were excluded from the study for various reasons and the study was completed with 16 patients. Twelve patients were diagnosed with ascending TTA and four were

diagnosed with descending TTA. Five of the patient group were female and all participants in control group were male. None of the patients had family history and nine of the patients had hypertension, two patients had pulmonary aortic hypertension, two patients had diabetes mellitus, one patient had myocardial infarction and five patients had obesity. The control group consisted of aortic tissues taken from nine patients who underwent surgery due to a heart disease with the exception of TTA. There were no cases of TTAs in the family of control subjects and five of the control subjects were diagnosed with hypertension, two were diagnosed with myocardial infarction, seven control subjects were diagnosed with diabetes mellitus and two had obesity. The clinical data of the subjects were obtained from the patient follow-up files and by questionnaire.

Gene expression analysis

The patients underwent repair surgery of TTA based on size bigger than 5.5 mm. For this purpose during the surgical operation for the treatment, 0.5–1 cm samples were taken from the aneurysmal tissues and were directly plunged into liquid nitrogen to protect the cell viability and freshness of the tissues. They were stored at -80°C until RNA isolation. The stored tissue samples were homogenized and total RNA isolation and cDNA synthesis were performed according to the kit protocol of Transcriptor High Fidelity cDNA Synthesis Kit (Roche). For the normalization of RNA quality and quantity in the samples, β -actin gene was used as a control gene. PCR conditions were performed at 95°C for 60 s (one cycle) after pre-denaturation, at 95°C for 10 s, at 55°C for 5 s, and at 72°C for 20 s (45 cycles). Quantitative reverse transcription polymerase chain reaction (Light-Cycler, Roche Diagnostics GmbH, Germany) was used for quantitation. RealTime Ready Single Assay (Roche) (113248 for FBN-1, 108165 for FN-1, 101221 for TIMP-3 and 143636 for β -actin) were used for quantitative PCR and mRNA levels of the related genes were determined using a 4 μL cDNA sample (Transcript ID: NM_000138.4 for FBN-1; Transcript ID: NM_212482.2 for FN-1; Transcript ID: NM_000362.4 for TIMP-3 and Transcript ID: NM_001101.4 for β -actin). All experiments were repeated twice. Following amplification, the program applied for melting curve analysis was 20 s at 95°C , 20 s at 40°C and 0 s (one cycle) at 85°C . Distilled water was used instead of cDNA as a negative control. Serial dilutions were prepared for standards and each dilution and samples were run in duplicate.

Statistical analysis

SPSS software (Version 17.0 for Windows; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The frequency, rate, average, and standard deviation values were used for the descriptive statistics of the data. $2^{-\Delta\Delta\text{CT}}$ method was used for the determining expression levels of the genes. Shapiro-Wilk was used for the test of normality and analysis of abnormally distributed values was performed with the Wilcoxon rank sum test. Student's t-test was used in normally distributed values. ANOVA, Fisher and χ^2 -tests were used for the presentation of demographic, characteristics and clinical data. $p\text{-Value} < 0.05$ was considered as statistically significant.

Results

The mean age of patients (TAA) ($n=16$) and controls ($n=9$) were 61.46 ± 13.87 , 66.44 ± 11 years, respectively. Body mass index, fasting blood glucose (FBG), total cholesterol (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) and other clinical data were given in Table 1. The values of FBG and ejection fraction (EF) were statically different between TAA and control groups ($p < 0.05$). The gene expression graph of the FBN-1, FN-1 and TIMP-3 gene was shown in Figure 1. The expression levels did not change according to the ascending or descending aortic aneurysm. Therefore, the results were evaluated as TTA. The value of the ΔCt , $\Delta\Delta\text{Ct}$, $2^{-\Delta\Delta\text{CT}}$ were given in Table 2 for each gene. The gene expression levels between the TTA and control groups were examined, only TIMP-3 gene was statically significant ($p=0.034$). Increased TIMP-3 expression levels were observed in 13 of the patients. Furthermore, increased TIMP-3 expression levels were associated with FBG, EF and creatinine, while decreased TIMP-3 expression levels ($n=3$) were associated with red blood cell (RBC) ($p < 0.05$). Although levels of expression of FBN-1 and FN-1 were not significant between the control and the TAA groups, decreased FBN-1 expression level was associated with creatinine ($p=0.034$). Increased FN-1 levels were associated with EF values ($p=0.022$), while increased or decreased FN-1 expression levels were statistically significant with pulse ($p < 0.05$). The statistical data's of each gene and clinical parameters were given in Tables 3 and 4.

Discussion

FBN-1, FN-1 and TIMP-3 gene expression levels in patients with TTA were analyzed, only TIMP-3 gene expression

Table 1: Demographic and clinical data of the subjects.

Parameters	Mean $\pm$ SD		p-Value
	TAA (n=16)	Control (n=9)	
Age (year)	61.46 $\pm$ 13.87	66.44 $\pm$ 11	0.437
BMI (kg/m ²)	29.52 $\pm$ 5.65	28.63 $\pm$ 6.01	0.269
Blood pressure (mmHg)	124.07 $\pm$ 17.95	132 $\pm$ 17.72	0.179
Pulse pressure (mmHg)	73.6 $\pm$ 11.50	71.33 $\pm$ 12.11	0.569
FBG (mg/dL)	102.66 $\pm$ 10.72	164.57 $\pm$ 98.31	0.001*
Total cholesterol (mg/dL)	196.08 $\pm$ 53.01	185.57 $\pm$ 56.11	0.663
LDL (mg/dL)	119.75 $\pm$ 34.52	116.25 $\pm$ 68.04	0.856
HDL (mg/dL)	52.75 $\pm$ 21	42.75 $\pm$ 13.35	0.585
TG (mg/dL)	195.45 $\pm$ 151.16	107.75 $\pm$ 21.70	0.089
RBC (cells/mm ³)	4,544,615 $\pm$ 616,801.1	4,044,944.4 $\pm$ 1,640,297	0.504
Creatinin (mg/dL)	0.842 $\pm$ 0.354	0.92 $\pm$ 0.18	0.232
EF (%)	60.67 $\pm$ 4.95	51.11 $\pm$ 10.24	0.010**

TAA, thoracic aortic aneurysm; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglyceride; RBC, red blood cell; EF, ejection fraction, *, p = 0.001; **, p = 0.010.

The parameters were given as Mean $\pm$ SD in thoracic aortic aneurysm and control groups.

Figure 1: FBN-1, FN-1 and TIMP-3 transcription levels are shown in patients with TAA.

Expression levels are given relative to β -actin expression (Mean $\pm$ SD) and presented as “fold change” respect to control. TAA, thoracic aortic aneurysm; FBN-1, fibrillin-1; FN-1, fibronectin-1; TIMP-3, tissue inhibitor of matrix metalloproteinase 3; *, p < 0.05; ns, not significant.

Table 2: FBN-1, FN-1 and TIMP-3 transcription levels (Mean $\pm$ SD) are shown in the group of TAA and control.

Gene	TAA (n=16)	Control (n=9)
FBN-1		
Δ Ct	10.05 $\pm$ 3.38	11.12 $\pm$ 2.82
$\Delta\Delta$ Ct		-1
$2^{-\Delta\Delta$ Ct		2
p-Value		0.234
FN-1		
Δ Ct	11.46 $\pm$ 2.5	11.7 $\pm$ 2.99
$\Delta\Delta$ Ct		-0.30
$2^{-\Delta\Delta$ Ct		1.23
p-Value		0.57
TIMP-3		
Δ Ct	8.66 $\pm$ 3.81	11.5 $\pm$ 2.64
$\Delta\Delta$ Ct		-3.19
$2^{-\Delta\Delta$ Ct		9.14
p-Value		0.034

TAA, thoracic aortic aneurysm; FBN-1, fibrillin-1; FN-1, fibronectin-1; TIMP-3, tissue inhibitor of matrix metalloproteinase 3. $2^{-\Delta\Delta$ Ct levels were found as “fold change” respect to control.

levels were found statically significant (p < 0.05). However, FBN-1 gene expression levels and creatinine levels were also found statically significant (p = 0.034). The pulse/min and EF were found related with FN-1 gene expression levels (p < 0.05). The expression levels of TIMP-3 were associated with FBG, RBC, creatinine and EF (p < 0.05).

The pathogenesis of TTA formation is multifactorial and includes medial degeneration, inflammatory reaction, cellular proliferation or matrix degradation. The

involvement of reactive oxygen derivatives has an important role for the pathogenesis of aneurysm formation. Reactive oxygen species, MMPs, angiotensin II, TGF- β , activate tumor necrosis factor act together. The MAPK/extracellular signal-regulating kinase (ERK) cascade is the one of the pathways involved in the formation of aneurysm. The inhibition of MAPK/ERK cascade by statin and ERK inhibitors has been shown in experimental models to reduce the formation of aneurysms [26]. It has been reported that defects in the FBN-1 gene cause excessive

Table 3: The association between FBN-1, FN-1 and TIMP-3 transcription levels relative to β -actin expression and clinical parameters are shown in patients with TAA.

	T	Df	Mean difference	95% CI	p-Value
FBN-1 expression					
Creatinin	2.373	13	0.38	(0.034–0.73)	0.034*
FN-1 expression					
Pulse/min	2.8	13	16.9	(3.84–29.96)	0.015*
EF	2.6	12.9	5	(0.86–9.14)	0.022*
TIMP-3 expression					
FBG	–3.032	4.936	–11.73	(–21.7 to 1.74)	0.029*
RBC	2.4	11	975,454	(98,524–1,852,385)	0.032*
Creatinin	2.6	12.4	0.29	(0.048–0.52)	0.022*
EF	3.6	12	5	(1.98–8.02)	0.004**

TAA, thoracic aortic aneurysm; FBN-1, fibrillin-1; FN-1, fibronectin-1; TIMP-3, tissue inhibitor of matrix metalloproteinase 3; FBG, fasting blood glucose; RBC, red blood cell; EF, ejection fraction; *, $p < 0.05$; **, $p < 0.01$.

Table 4: The statistical association between FBN-1, FN-1 and TIMP-3 decreased/increased transcription levels relative to β -actin expression and clinical parameters are shown in patients with TAA.

	n	Mean	Standard deviation	Mean standard error	p-Value
FBN-1 expression					
Creatinin					
Decreased	7	1.07	0.35	0.14	0.034*
Increased	9	0.69	0.27	0.09	0.052
FN-1 expression					
Pulse/min					
Decreased	6	92.8	10.35	4.63	0.015*
Increased	10	75.9	11.33	3.58	0.018*
EF					
Decreased	6	64	2.24	1	0.062
Increased	10	59	5.16	1.6	0.022*
TIMP-3 expression					
FBG					
Decreased	3	92.5	3.54	2.5	0.157
Increased	13	104.23	10.65	2.95	0.029*
RBC					
Decreased	3	5,370,000	381,837.6	270,000	0.032*
Increased	13	4,394,545.45	530,025.73	159,808.7	0.102
Creatinin					
Decreased	3	1.09	0.057	0.04	0.306
Increased	13	0.8	0.37	0.102	0.022*
EF					
Decreased	3	65	0	0	0.194
Increased	13	60	5	1.38	0.004**

TAA, thoracic aortic aneurysm; FBN-1, fibrillin-1; FN-1, fibronectin-1; TIMP-3, tissue inhibitor of matrix metalloproteinase 3; FBG, fasting blood glucose; RBC, red blood cell; EF, ejection fraction; *, $p < 0.05$; **, $p < 0.01$.

TGF- β signaling first and then leading to weakening of the aortic wall with increased tissue degradation [16, 27]. It has recently been shown that FBN-1 was associated with TGF- β binding protein-1. When FBN-1 is mutated, TGF- β can no longer be stabilized and resulting in increased

TGF- β activation. Therefore, FBN-1 is important for regulatory control of TGF- β [27]. In our study, FBN-1 expression levels were observed two fold higher in patient groups ($p > 0.05$), but we found decreased FBN-1 levels in seven of the 16 patients. Decreased FBN-1 levels were related

increased creatinin levels ($p=0.034$). The decrease in the expression levels of fibrillin may have led to an increase in creatinine levels as a result of the degeneration of the aortic wall. Genetic mutations in *FBN-1* can lead the MFS [13, 14] and can cause decreasing fibrillin microfibrils providing elasticity to the tissue. Thus, maintaining the structure and integrity of the ECM, fibrillin is important [28]. However, because of our patients had no family history, TAA may not be caused by *FBN-1* mutations and a genetic background showing familial transition. That can explain the not decreased levels of the *FBN-1* gene expression levels. An increase in fibrillin levels may be observed to prevent loss of elastin after aneurysm even though half of the patients had decreased *FBN-1* levels.

FN has different variants due to a single genetic alternative clipping/splicing process. The soluble form in plasma is dimeric and lacks of the alternative spliced EDA (exon33) and EDB (exon 25). The multimeric forms found in tissue include EDA or EDB. The EDA and EDB sequences show low homology. Thus, it is thought that these domains have different functions. The exact functions of EDA and EDB on the vessel wall are not known yet [29]. In our study, we evaluated EDA and EDB domains together due to sample numbers and no statistically significant difference was found between study and control samples in *FN-1* gene expression levels. Ten of the 16 patients had increased *FN-1* expression levels. Increased *FN-1* levels were found significantly related with decreased EF levels ($p=0.022$). *FN-1* levels were also found associated with pulse rate/min ($p<0.05$).

MMPs are endopeptidases that degrade ECM proteins such as collagen, FN, and lamin. Activities are regulated by TIMPs [19]. MMPs and their tissue inhibitors (TIMPs) regulate the formation of the matrix in the heart [30]. As a result of the studies performed in ruptured aortic tissue; increased expression and activities of MMPs play a fundamental role in this disease. Inhibition of MMPs is thought to abolish the aortic damage [31]. Differences in MMPs and TIMPs expression are the basis for the development of ascending aortic dilatation [22]. A study found that *TIMP-3* expression levels in smooth muscle cells decrease in patients with TTAs (relative expression value control: 663, tricuspid aortic valves smooth muscle cells: 222; $p=0.013$) [32]. Another study showed the critical role of *TIMP-3* in constructive vascular remodeling in *TIMP-3*-deficient mice. They also suggested that targeted overexpression of *TIMP-3* may prevent aneurysm formation or controlling its expansion [33]. In the study conducted by Tsarouhas et al. [22], transcription levels of *TIMP-1* and *TIMP-4* genes decreased when compared to controls, but it was not statistically significant. In

the same study, high expression levels for the *TIMP-3* gene were found significantly elevated in aortic aneurysm (1.74) compared to controls (1.25) ($p=0.045$) [22]. High expression levels of *TIMP-3* have been found to be positively correlated with increased MMP activity. In the study, it was indicated that the ratio of MMP/TIMP would be a better guide for the investigation of MMP activity and degradation of the aortic ECM, rather than seeking each molecule increased or decreased [22]. Jones et al. studied that MMPs/TIMPs gene expression in primary murine aortic fibroblasts isolated from normal and TAA-induced mice. They found increased gene expression of *MMP-2* [relative fold (rf):6.54, $p=0.0055$] and decreased gene expression of *MMP-3* (rf:0.41, $p=0.36$), *MMP-9* (rf:0.09, $p=0.048$), *TIMP-1* (rf:1.41, $p=0.24$), *TIMP-2* (rf:1.11, $p=0.58$) and *TIMP-3* (rf:0.33, $p=0.15$) in TTA fibroblasts compared with normal fibroblasts [34]. Although increased levels of MMPs are expected in the TTA, low levels of MMPs gene expression were observed. TIMPs levels may elevate due to an increase in MMPs expression levels or, in contrast, a decrease in TIMPs expression levels can cause increasing of MMPs levels. Because of *TIMP-3* inhibits a broad spectrum of TIMP [35], its expression can be affected by a large number of MMP. In our study, the elevated *TIMP-3* level in the TAA group may be increased in order to balance the increased MMPs levels. However, since we did not measure the MMPs levels, we have no evidence to determine this result. We examined the fold change values of 16 patients to investigate of expression of *TIMP-3* gene. Decreased expression levels were detected in three patients and increased expression levels were found in 13 patients. *TIMP-3* levels were determined nine fold higher in patients ($p=0.034$). *TIMP-3* expression levels were also found associated with FBG, RBC, creatinine and EF ($p<0.05$). There are various results and publications opposite to each other, particularly regarding *TIMP-3* protein. This is interference in the correct treatment for the survival and the high increase in *TIMP-3* expression in aneurysm specimens indicates substantially protease inhibition in the aortic aneurysm.

According to the results of this clinical trial, we concluded that *TIMP-3* gene might play a role in the formation of TTA. The major limitation of our study was the number of participants and study groups consisted of both sexes. Furthermore, expression levels were not confirmed at the protein levels and were not shown immunohistochemically in addition to lacking of serum samples, we could not study the serum levels of *FBN-1* and *TGF- β* . It is necessary to confirm our results with a larger number of samples.

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References

- Huang J, Davis EC, Chapman SL, Budatha M, Marmorstein LY, Word RA, et al. Fibulin-4 deficiency results in ascending aortic aneurysms: a potential link between abnormal smooth muscle cell phenotype and aneurysm progression. *Circ Res* 2010;106:583–92.
- Pape LA, Awais M, Woznicki EM, Suzuki T, Trimarchi S, Evangelista A, et al. Presentation, diagnosis, and outcomes of acute aortic dissection: 17-year trends from the International Registry of Acute Aortic Dissection. *J Am Coll Cardiol* 2015;66:350–8.
- Aziz F, Penupolu S, Alok A, Doddi S, Abed M. Peripartum acute aortic dissection: a case report & review of literature. *J Thorac Dis* 2011;3:65–7.
- Milewicz DM, Regalado ES, Guo DC. Treatment guidelines for thoracic aortic aneurysms and dissections based on the underlying causative gene. *J Thorac Cardiovasc Surg* 2010;140:S2–4; discussion S45–51.
- Melvinsdottir IH, Lund SH, Agnarsson BA, Sigvaldason K, Gudbjartsson T, Geirsson A. The incidence and mortality of acute thoracic aortic dissection: results from a whole nation study. *Eur J Cardiothorac Surg* 2016;50:1111–7.
- Leitman IM, Suzuki K, Wengrofsky AJ, Menashe E, Poplawski M, Woo KM, et al. Early recognition of acute thoracic aortic dissection and aneurysm. *World J Emerg Surg* 2013;8:47.
- Spin JM. Gene mutations and familial thoracic aortic aneurysms: a walk on the mild side. *Circ Cardiovasc Genet* 2011;4:4–6.
- Dingemans KP, Teeling P, Lagendijk JH, Becker AE. Extracellular matrix of the human aortic media: an ultrastructural histochemical and immunohistochemical study of the adult aortic media. *Anat Rec* 2000;258:1–14.
- Sabatier L, Chen D, Fagotto-Kaufmann C, Hubmacher D, McKee MD, Annis DS, et al. Fibrillin assembly requires fibronectin. *Mol Biol Cell* 2009;20:846–58.
- Humphrey JD, Schwartz MA, Tellides G, Milewicz DM. Role of mechanotransduction in vascular biology: focus on thoracic aortic aneurysms and dissections. *Circ Res* 2015;116:1448–61.
- Wilson JS, Baek S, Humphrey JD. Importance of initial aortic properties on the evolving regional anisotropy, stiffness and wall thickness of human abdominal aortic aneurysms. *J R Soc Interface* 2012;9:2047–58.
- Humphrey JD, Milewicz DM, Tellides G, Schwartz MA. Cell biology. Dysfunctional mechanosensing in aneurysms. *Science* 2014;344:477–9.
- Lindsay ME, Dietz HC. The genetic basis of aortic aneurysm. *Cold Spring Harb Perspect Med* 2014;4:a015909.
- Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. *Science* 2006;312:117–21.
- Paloschi V, Kurtovic S, Folkersen L, Gomez D, Wågsäter D, Roy J, et al. Impaired splicing of fibronectin is associated with thoracic aortic aneurysm formation in patients with bicuspid aortic valve. *Arterioscler Thromb Vasc Biol* 2011;31:691–7.
- Booher AM, Eagle KA. Diagnosis and management issues in thoracic aortic aneurysm. *Am Heart J* 2011;162:38–46.
- Giusti B, Sticchi E, De Caro R, Magi A, Nistri S, Pepe G. Genetic bases of bicuspid aortic valve: the contribution of traditional and high-throughput sequencing approaches on research and diagnosis. *Front Physiol* 2017;8:612.
- Nistri S, Basso C, Thiene G. [Bicuspid aortic valve]. *G Ital Cardiol (Rome)* 2012;13:25–37.
- Hopps E, Caimi G. Matrix metalloproteinases in metabolic syndrome. *Eur J Intern Med* 2012;23:99–104.
- Murphy G. Tissue inhibitors of metalloproteinases. *Genome Biol* 2011;12:233.
- Brew K, Nagase H. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochim Biophys Acta* 2010;1803:55–71.
- Tsarouhas K, Soufla G, Apostolakis S, Zaravinos A, Panagiotou M, Khoury M, et al. Transcriptional regulation of TIMPs in ascending aorta aneurysms. *Thromb Res* 2010;126:399–405.
- Shen Y, Winkler IG, Barbier V, Sims NA, Hendy J, Lévesque JP. Tissue inhibitor of metalloproteinase-3 (TIMP-3) regulates hematopoiesis and bone formation in vivo. *PLoS One* 2010;5:e13086.
- Sulkava M, Raitoharju E, Mennander A, Levula M, Seppälä I, Lyytikäinen LP, et al. Differentially expressed genes and canonical pathways in the ascending thoracic aortic aneurysm – the Tampere Vascular study. *Sci Rep* 2017;7:12127.
- Alajbegovic A, Holmberg J, Albinsson S. Molecular regulation of arterial aneurysms: role of actin dynamics and microRNAs in vascular smooth muscle. *Front Physiol* 2017;8:569.
- Attenhofer Jost CH, Greutmann M, Connolly HM, Weber R, Rohrbach M, Oxenius A, et al. Medical treatment of aortic aneurysms in Marfan syndrome and other heritable conditions. *Curr Cardiol Rev* 2014;10:161–71.
- Halushka MK. Single gene disorders of the aortic wall. *Cardiovasc Pathol* 2012;21:240–4.
- Davis MR, Summers KM. Structure and function of the mammalian fibrillin gene family: implications for human connective tissue diseases. *Mol Genet Metab* 2012;107:635–47.
- van Keulen JK, de Kleijn DP, Nijhuis MM, Busser E, Velema E, Fijnheer R, et al. Levels of extra domain A containing fibronectin in human atherosclerotic plaques are associated with a stable plaque phenotype. *Atherosclerosis* 2007;195:e83–91.
- Guenzinger R, Lahm H, Wottke M, Lange R. Role of metalloproteinases and tissue inhibitors of metalloproteinases during cardiopulmonary bypass in rats. *ASAIO J* 2012;58:204–11.
- Zhang X, Shen YH, LeMaire SA. Thoracic aortic dissection: are matrix metalloproteinases involved? *Vascular* 2009;17:147–57.
- Blunder S, Messner B, Aschacher T, Zeller I, Türkcan A, Wiedemann D, et al. Characteristics of TAV- and BAV-associated thoracic aortic aneurysms – smooth muscle cell biology, expression profiling, and histological analyses. *Atherosclerosis* 2012;220:355–61.

33. Basu R, Fan D, Kandalam V, Lee J, Das SK, Wang X, et al. Loss of Timp3 gene leads to abdominal aortic aneurysm formation in response to angiotensin II. *J Biol Chem* 2012;287:44083–96.
34. Jones JA, Zavadzkas JA, Chang EI, Sheats N, Koval C, Stroud RE, et al. Cellular phenotype transformation occurs during thoracic aortic aneurysm development. *J Thorac Cardiovasc Surg* 2010;140:653–9.
35. Kashiwagi M, Tortorella M, Nagase H, Brew K. TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5). *J Biol Chem* 2001;276:12501–4.