A novel association between TGFβ1 and ADAMTS4 in coronary artery disease: A new potential mechanism in the progression of atherosclerosis and diabetes

Safiye Uluçay, Fethi Sırrı Çam, Muhammed Burak Batır, Recep Sütçü[1] Özgür Bayturan, Kadir Demircan[2]*

Departments of Medical Genetics and *Cardiology, Faculty of Medicine, Celal Bayar University; Manisa-*Turkey*
[1]Department of Biochemistry, Faculty of Medicine, Katip Çelebi University; İzmir-*Turkey*
[2]Department of Medical Biology, Faculty of Medicine, Turgut Özal University; Ankara-*Turkey*

ABSTRACT

Objective: Coronary artery disease is characterized by atherosclerosis in the vessel wall. Recently, it has been thought that increasing LDL-binding capacity of subendothelial proteoglycan fragments that are formed by protease activity can be responsible for the initiation of atherosclerosis. ADAMTS4 is a member of the versican-degrading proteinases. In vitro studies demonstrated that TGFβ inhibits the expression of ADAMTS4 in macrophages. In this study, we aimed to investigate the role and association between TGFβ1 and ADAMTS4 in coronary artery disease.

Methods: A total of 84 cases with atheroma plaque and 72 controls without plaque were analyzed. The severity of disease was determined by Gensini score. TGFβ1 gene polymorphisms were genotyped by the PCR-RFLP method. TGFβ1 and ADAMTS4 serum levels were measured by ELISA method. Statistical analyses of genotypes and their relationship with serum levels were performed by chi-square, student t test and ANOVA.

Results: ADAMTS4 levels were higher in cases compared with controls ($p<0.05$). In the patient group, ADAMTS4 levels were higher than in controls and correlated with TGFβ1 serum levels ($r=0.29$; $p<0.05$) and severity of disease ($r=0.20$; $p<0.05$). The TGFβ1 gene CCA haplotype was associated with 3.3-fold increase in coronary artery disease ($OR=3.26$ 95% CI 1.22-8.68; $p<0.05$). Unexpectedly, ADAMTS4 serum levels were also higher in diabetic cases ($p=0.05$).

Conclusion: This study has demonstrated that ADAMTS4 may be responsible for the pathogenesis of atherosclerosis. This is the first report about the association between ADAMTS4 and TGFβ1 serum levels in the progression of atherosclerosis in CAD. Furthermore, it is seen that TGFβ1 haplotype can cause a genetic susceptibility to CAD in the Turkish population. To our knowledge, this is also the first report suggesting higher serum ADAMTS4 levels in diabetic patients. *(Anatol J Cardiol 2015; 15: 823-9)*

Keywords: atherosclerosis, extracellular matrix, ADAMTS4, TGFβ1 gene polymorphism, diabetes

Introduction

Coronary artery disease (CAD), which is responsible for a large majority of cardiovascular diseases, has high mortality and morbidity. In the vast of majority of cases, subintimal thickening, named 'atherosclerosis,' is responsible for the pathogenesis of coronary artery disease.

Atherosclerosis is a complex and heritable disease in the vessel walls that develops over many years. It is characterized by low-density lipoprotein deposition in the arterial wall, a process that is stimulated by environmental and genetic factors (1). In 1995, Williams et al. (2) hypothesized the 'Response-to-Retention Hypothesis,' in which subendothelial retention of atherogenic lipoproteins can be responsible for the initiation of atherosclerosis. In this retention, subintimal extracellular matrix proteoglycans, especially versican, have been charged (3). In vitro studies showed that versican stimulates cell adhesion, cell proliferation, and cell migration, which are important processes in atherosclerosis (4).

Recently, it was shown that proteoglycan fragments that are formed by protease activity accumulate in normal and diseased vessel walls (5). A disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) is a member of the versican-degrading proteinases (5). After Sandy et al. (6) showed that versican fragments that are formed by ADAMTS4 proteinase accumulate in human aorta, in vitro studies showed that these fragments stimulate vascular smooth muscle cell migration (VSMC) (7). So, it has been thought that these fragments can be

824 Uluçay et al.
TGFβ1 and ADAMTS4 in coronary disease

Anatol J Cardiol 2015; 15: 823-9

biologically active fragments. Also, in clinical studies, enhanced serum ADAMTS4 levels were found in CAD (8, 9). ADAMTS4 is an inflammation-regulated enzyme, and its expression is regulated by cytokines (10). In vitro studies showed that ADAMTS4 expression is inhibited by transforming growth factor β (TGFβ) (11). TGFβ1 is the most abundant isoform in the healthy blood vessel wall (12).

TGFβ1, responsible for the synthesis of extracellular matrix, cell growth, cell differentiation, cell migration, and apoptosis, is a multifunctional cytokine and is synthesized by endothelial cells, VSMCs, and myofibroblasts in the cardiovascular system (13, 14). The role of TGFβ1 in atherosclerosis is controversial. First, it was thought that TGFβ1 was an atherogenic cytokine by stimulating the production of lipoprotein-trapping proteoglycans, but in other studies, it has been thought that TGFβ1 is an antiatherogenic and protective cytokine because of its antiatherogenic functions, like inhibition of VSMC and leukocyte cell proliferation, migration, and vascular endothelial adhesion molecules (12, 15).

This study aimed to investigate the role of TGFβ1 and ADAMTS4 in CAD. For this purpose, we investigated TGFβ1 and ADAMTS4 serum levels and the functional TGFβ1 gene polymorphisms rs1800469, rs1800470, and rs4803455 polymorphism.

Methods

Study design

In this case control study, we analyzed functional TGFβ1 polymorphisms with TGFβ1 serum levels and ADAMTS4 serum levels through coronary artery disease and healty subjects.

Study population

In this study, Turkish patients, 70 men and 86 women, who were referred to coronary angiography for the evaluation of suspected coronary artery disease from December 2012 to May at Celal Bayar University Hospital in Manisa, were enrolled. Referral to coronary angiography was based on a clinical indication according to the current guidelines (16). As a result of coronary angiography, 84 cases who had atheroma plaque were included into the patient group, and 72 cases who did not have atheroma plaque were included into the control group. Demographic features and atherosclerotic risk factors were recorded for all participants. In the patient group, diseased vessel number and percentage of lesion were determined using the Gensini score for severity of disease. Katip Çelebi University Faculty of Medicine's Ethical Committee approved the study protocol, and written informed consent was obtained from each subject.

TGFβ1 genotyping

Genomic DNA was extracted from peripheral blood using the commercial İnvitrogen Genomic DNA extraction kit (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions and stored at -20°C. The genotypes of rs1800469,

Table 1. Primers, enzymes, and products for each polymorphism

Polymorphism	Enzyme	Primers	PCR product
rs1800469	DdeI	F:5′-ACAGGTGTCTGCCTCCTGAC-3′	223 bp
		R: 5′-CCTCTTTCTCTGGTGACCCA-3′	
rs1800470	MspA1I	F: 5′-TTCAAGACCACCCACCTTCT-3′	368 bp
		R: 5′-ATCGACATGGAGCTGGTGAA-3′	
rs4803455	MluCI	F:5′-GGCTCTAGAAGTGGAAATCTTG-3′	460 bp
		R: 5′-CAGGGTGTCAAATTTGCAGAAC-3′	
bp - base pair; PCR - polymerase chain reaction			

rs1800470, and rs4803455 were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers, enzymes, and products for each polymorphism are shown in Table 1. The PCR was performed in a 25 µL reaction containing 150 ng DNA, 10x PCR buffer, 2.5 mM MgCl$_2$, 20 µM dNTPs, forward primer (10 pmoL/µL), reverse primer (10 pmoL/µL), and 5U/µL hot start Taq polymerase. Amplification conditions were set up as follows: an initial activation step of 94°C for 15 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 60°C for 45 sec, extension at 72°C for 1 min and 45 sec, and a final extension step at 72°C for 10 min. PCR products were digested by restriction enzyme (showed in Table 1) at 37°C overnight. For primers, the online software primer design program Primer3Plus (http://www.bio-informatics.nl/cgi-bin/primer3plus/primer3plus.cgi) was used.

Quantification of TGFβ1 and ADAMTS-4 serum levels

Serum samples were used in this study. The blood samples were collected and allowed to clot before centrifugation. After centrifugation, serum was removed and stored at -20°C. Enzyme-linked immunosorbent assay (ELISA) was performed for measuring serum levels using commercially available kits [USCN Life Science (USCNK)], following the manufacturer's instructions.

Severity of disease

The Gensini score was used to determine the severity of CAD in the CAD group, and it was defined according to stenosis severity as 1 point for <25% stenosis, 2 points for 26% to 50% stenosis, 4 points for 51% to 75% stenosis, 8 points for 76% to 90% stenosis, and 32 points for total occlusion. The calculated scores were thereafter multiplied according to factors that defined the importance of a stenosis site.

Statistical analysis

Statistical Package for Social Sciences (SPSS), version 15 for (version 15.0, SPSS, Chicago, IL, USA) was used for data analysis. Comparison of continuous variables was performed using student t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. One-way analysis of variance was used to test the differences

Anatol J Cardiol 2015; 15: 823-9

Uluçay et al.
TGFβ1 and ADAMTS4 in coronary disease *825*

of means for continuous variables, and Pearson's χ^2 test was performed to compare the categorical variables between cases and controls. Correlation analysis tests were used for interdependence of the variables. Receiver operating characteristic analysis (ROC) was used to determine the sensitivity and specificity of serum ADAMTS4 levels. SHEsis online software (http://analysis.bio-x.cn/myAnalysis.php, The Bio-X Research Institute of Shanghai Jiao Tong University, Shanghai, China) was used to construct the haplotypes for the identified polymorphisms. A p value <0.05 was considered statistically significant.

Results

Clinical characteristics are presented for 156 participants in Table 2. Diabetes mellitus, hypertension, and hyperlipidemia were significantly more common in the patient group when compared with the control group.

Allele frequencies of cases and controls are summarized in Table 3. There was no difference between groups for genotype and allele frequencies for each polymorphism.

To determine the possibility of the combined effects of the three TGFβ1 polymorphisms, haplotype analysis was used to determine TGFβ1 haplotypes. The TGFβ1 CCA haplotype was significantly more common in patients than in controls and was associated with a 3.3-fold increase in CAD. In Table 4, the combined genotype frequencies are presented. For the rs1800469 and rs1800470 polymorphisms, the CC haplotype was associated with 2.3-fold increase in CAD.

TGFβ1 serum levels measured 19.12±12.2 ng/mL in atherosclerotic patients and 20.62±15.5 ng/mL in controls. Although patients' mean serum TGFβ1 levels were lower than in controls, there was no significant difference between groups (p=0.50), and serum TGFβ1 levels were not associated with genotypes.

ADAMTS4 serum levels measured 203.4±128.2 ng/mL in patients and 105.4±82.5 ng/mL in controls. ADAMTS4 serum levels were significantly higher in patients (p=0.001). The severity of disease (Gensini score) correlated with ADAMTS4 serum levels (r=0.20; p=0.012).

The receiver operating characteristic ROC curve analysis showed that ADAMTS4 serum levels of 101.130 ng/mL could predict CAD with 76.2% sensitivity and 67.7% specificity (Fig. 1).

For patients, TGFβ1 serum levels correlated with ADAMTS4 serum levels (r=0.29; p=0.007). In diabetic patients, TGFβ1 serum levels correlated with ADAMTS4 serum levels but not significantly (r=0.26, p=0.08) (Fig. 2).

In diabetic patients, ADAMTS4 serum levels were significantly higher than in non-diabetic participants. The ADAMTS4 serum levels of diabetics with atheroma plaque were significantly higher than in diabetics without atheroma plaque (Table 5). There was no significant difference between groups for TGFβ1 serum levels (p=0.65).

ADAMTS4 - A disintegrin and metalloproteinase with thrombospondin motifs 4.

Table 2. Clinical characteristics of cases and controls

Features	Cases (n=84)	Controls (n=72)	P
Age, years	60.25±10.11	51.80±7.9	10^{-3}
Male	44 (52.4%)	26 (36.1%)	10^{-3}
Female	40 (47.6%)	46 (63.9%)	10^{-3}
Diabetes mellitus	32 (38.1%)	11 (15.3%)	10^{-3}
Hypertension	40 (47.6%)	25 (34.7%)	10^{-3}
Hyperlipidemia	35 (41.7%)	12 (16.7%)	10^{-3}
Family history	24 (28.6%)	15 (20.8%)	NS
Smoking	19 (22.6%)	14 (19.4%)	NS

NS - no significance, p>0.05
A *P* values for chi-square, Student t-test or Mann-Whitney U tests

Table 3. Allele frequencies of cases and controls for each polymorphism

Polymorphism	Allele	Cases, %	Controls, %	P
rs1800469	C	54.2	50	0.46
	T	45.8	50	
rs1800470	T	50	55.6	0.32
	C	50	44.4	
rs4803455	A	38.7	33.3	0.32
	C	61.3	66.7	

Calculated by chi-square test

Table 4. Haplotype frequencies of TGFβ1 polymorphisms

Haplotype	Cases	Controls	P	OR (95% CI)
CCC	14.9 (0.8%)	8.38 (0.058%)	0.306	1.57 (0.65-3.71)
CCA	19.12 (11%)	5.46 (0.38%)	*0.013	*3.26 (1.22-8.68)
CTC	19.02 (11.3%)	16.91 (11.7%)	0.908	0.96 (0.47-1.9)
CTA	37.95 (22%)	41.24 (28%)	0.221	0.72 (0.43-1.21)
TCC	43.67 (26%)	50.16 (34.8%)	0.089	0.65 (0.40-1.06)
TTC	25.41 (15%)	20.55 (14.3%)	0.829	0.65 (0.40-1.06)

*P<0.05
CI - confidence interval; OR - odds ratio
A *P* value for chi-square

Table 5. ADAMTS4 serum levels for diabetic patients

Serum levels, ng/mL	Diabetics (n=43)	Non-diabetics (n=113)	P
ADAMTS4	187.771	146.918	*0.05
	Diabetics with atheroma (n=32)	**Diabetics without atheroma (n=11)**	**P**
	212.17	116.79	*0.03

*p<0.05
ADAMTS4 - A disintegrin and metalloproteinase with thrombospondin motifs 4
Calculated by ANOVA

Discussion

Herein, we showed that ADAMTS4 serum levels were significantly higher in atherosclerotic patients. This result suggests
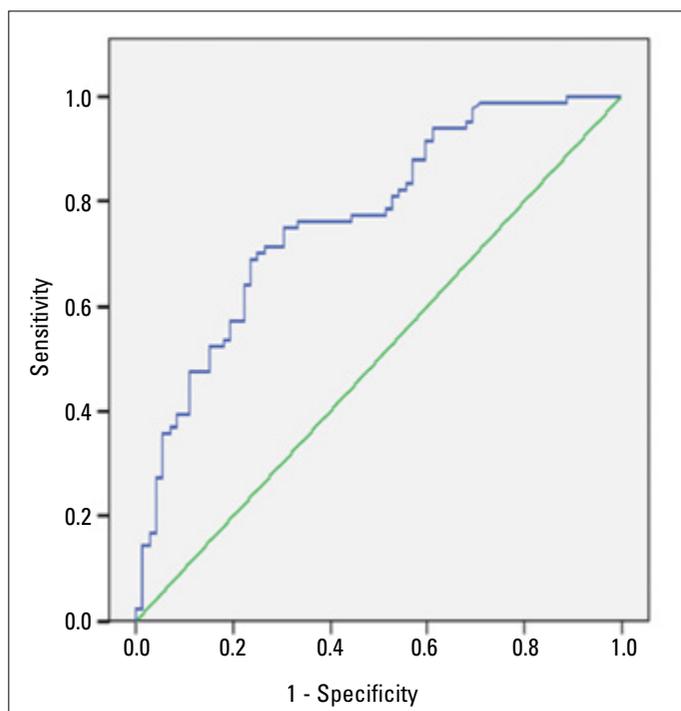
826 Uluçay et al.
TGFβ1 and ADAMTS4 in coronary disease

Anatol J Cardiol 2015; 15: 823-9

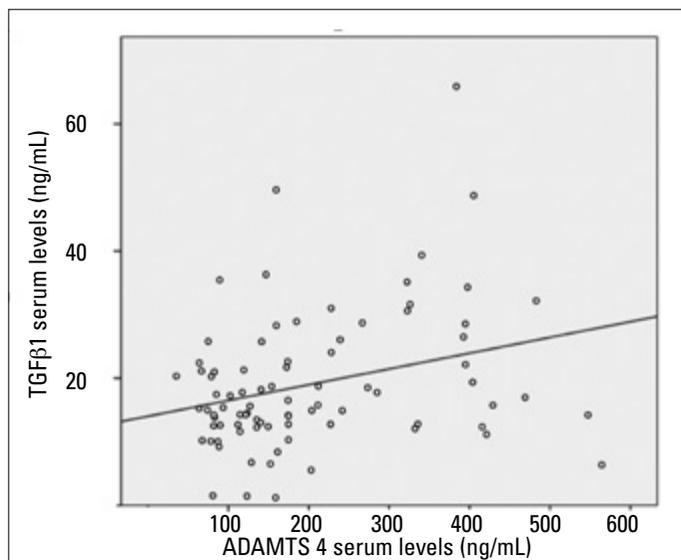**Figure 1. ROC curve analysis of ADAMTS4 serum levels**

Figure 2. Scattered point diagram of correlation between TGFß1 and ADAMTS4 levels

that ADAMTS4 serum levels are associated with angiographic presence and severity of CAD. We also showed for the first time an association between TGFβ1 and ADAMTS4 serum levels in atherosclerotic patients.

Recently, it was thought that remodeling in the subintimal extracellular matrix in the vessel wall plays an important role in the formation of atherosclerosis, especially in lipid retention and cell migration (4, 17, 18). Many studies have implicated the importance of extracellular matrix-degrading proteinases, such as matrix metalloproteases (MMPs), cysteine proteinases, and

serine proteinases, and also the importance of plasminogen activator and plasminogen in matrix remodeling and vascular smooth muscle cell migration (8). ADAMTS family members are non-membrane-bound enzymes that are able to interact with components of the extracellular matrix and degrade extracellular matrix proteoglycans (7). From the members of ADAMTS, it was known that ADAMTS1, 4, 5, and 9 have been reported to cleave versican (5). It was shown that ADAMTS1 and ADAMTS4 are the most abundant proteinases in atherosclerotic plaque (7). In 2013, ADAMTS4 was identified as a potential pathogenic factor for plaque instability in mice and was validated in human plaques (19).

There are only a few studies about the effects of ADAMTS4 serum levels in atherosclerotic patients. Chen et al. (8) demonstrated elevated ADAMTS4 serum levels in CAD and a correlation with severity of disease, and they suggested that ADAMTS4 might serve as an independent factor for predicting CAD. Zha et al. (9) showed an association between ADAMTS4 serum levels and plaque destabilization. According to our results, we suggest that ADAMTS4 can have an active role in the progression of atherosclerosis and that ADAMTS4 serum levels can be used for prediction and severity of CAD.

In vitro studies showed that ADAMTS4 expression was increased following monocyte-macrophage differentiation during atherogenesis (7). The induction of ADAMTS4 expression following monocyte-macrophage differentiation is thought to be mediated through a secondary signal (10). Recently, Salter et al. (11) demonstrated that ADAMTS4 expression in macrophages is inhibited by TGFβ through Smads, p38 mitogen-activated protein kinase, and the c-Jun pathway.

The expression of TGFβ1 is under genetic control and stringently regulated (20). TGFβ1 is coded by the TGFβ1 gene, which is localized in the 19q13.1 locus and composed of 7 exons. Especially, it has been known that promoter region polymorphisms regulate TGFβ1 expression in various cell types. It has been shown that the TGFβ1 gene promoter site includes nearly 2.6 kb of DNA sequence former to translation beginning point (21). We investigated two functional TGFβ1 polymorphisms (rs1800469, rs1800470) and an intronic region polymorphism (rs4803455), which is in linkage disequilibrium (LD) with the rs1800470 polymorphism, and the association between serum TGFβ1 levels with genotypes. rs1800469 (-509C-T, c.-1347C>T) is a polymorphism localized to the proximal negative regulatory site, which is known to affect TGFβ1 expression (21). rs1800470 (+869T-C, c.+29T>C, p.Pro10Leu) is also a polymorphism localized to exon 1, which has a proline instead of leucine in codon 10 and functions as a part of a peptide signal sequence. It is thought that it changes the serum concentration by affecting the transport of TGFβ1 to the endoplasmic reticulum, which is synthesized as a preprotein (22). We failed to detect an association of these TGFβ1 polymorphisms between groups, and there was no difference in TGFβ1 serum levels between groups or genotypes. Similarly, no significantly association was found for the

Anatol J Cardiol 2015; 15: 823-9

Uluçay et al.
TGFβ1 and ADAMTS4 in coronary disease   *827*

development of atherosclerosis and the destabilization of plaque in other studies (22, 23), but Koch et al. (24) showed that the rs1800469 T allele and rs1800470 C allele cause genetic susceptibility to destabilization of plaque (25). For the rs4803455 polymorphism, Deng et al. (26) demonstrated an association between carotid plaque with this polymorphism, but our results suggest that this polymorphism is not associated with coronary atherosclerosis. But, we demonstrated that the TGFβ1 CCA haplotype is significantly associated with CAD in our study. But, we could not detect how TGFβ1 serum levels were affected by these haplotypes. This result suggests that alleles of TGFβ1 may be susceptible to CAD, but this finding must be confirmed with more patients in our population.

In this study, we could not show TGFβ1 serum level differences for the presence and progression of atherosclerosis. Tashiro et al. (27) demonstrated that reduced plasma level of TGFβ1 was significantly found in atherosclerotic patients and that TGFβ1 could be regarded as a stable prognostic marker of CAD. We showed no significant association between serum TGFβ1 levels with CAD. This result may arise from the effect of different pathophysiological stages of CAD on TGFβ1 serum levels, and circulating TGFβ1 levels may not reflect the vascular interstitial and circulating active TGFβ1 levels (28). It has been shown by Grainger et al. (29) TGFβ1 active type is at lower concentrations in advanced atherosclerotic patients. Chen et al. (30) showed that serum TGFβ1 concentrations are statistically significantly higher in patients with acute myocardial infarction (MI when compared to stable and unstable angina patients).

TGFβ1 regulates a range of functions, and the pleiotropic effects of TGFβ1 are mediated through several receptors (31). TGFβ1 and its signaling pathway have been researched in the pathogenesis of atherosclerosis because of the bipotential effects of TGFβ1 in the vessel wall (32). Thus, the role of other molecules functioning in the signaling pathway in the pathogenesis of disease is being investigated (30).

Our results suggested that TGFβ1 and ADAMTS4 serum levels increased together during the progression of atherosclerosis. Contrary to in vitro studies, synchronically high plasma concentrations of TGFβ1 and ADAMTS4 for the progression of disease show us there may be different TGFβ1 signaling mechanisms in the pathogenesis under in vivo conditions. We hypothesize that reduced TGFβ1 signaling may result in upregulation of TGFβ1 serum levels. In the literature, this condition was called the 'TGFβ1 paradox,' which has noted anomalies between elevated levels of TGFβ1 versus a marked decrease in one or more of the TGFβ1 responses (32). In asthma, it was known that although the pulmonary levels of TGFβ1 increase, the immunosuppressive effect of TGFβ1 decreases (33). This condition may result from TGFβ1 being able to activate its own mRNA expression and increase its own secretion because of reduced TGFβ1 signaling (31).

Recently, reduced TGFβ1 signaling with aging was shown in VSMCs (32, 34). We thought that aging or other factors that affect TGFβ1 signaling may be responsible for the progression of disease, and the inhibition effect of TGFβ1 on ADAMTS4 expression may reduce and progress the disease. With further studies, the impact of reduced TGFβ1 signaling pathway on the vessel wall must be investigated. This area may become a target for preventive treatment in CAD for the progression of disease. For the association between TGFβ1 and ADAMTS4, tissue expression studies should be planned to evaluate ADAMTS4 expression depending on TGFβ1 serum levels.

In this study, we first demonstrated that diabetic patients show higher serum ADAMTS4 levels compared with non-diabetic participants. Excess accumulation of vascular extracellular matrix (ECM) is an important pathological process in cardiovascular diseases, including diabetes-associated atherosclerosis (35). However, the underlying molecular mechanisms have not been fully understood. Recently, genome wide association studies about ADAMTS9, which has similar functions as ADAMTS4 on extracellular matrix, showed that ADAMTS9 expression tended to be downregulated by high glucose in diabetes and that the ADAMTS9 gene is a genetic susceptibility gene for diabetes (5, 36). To our knowledge, there is no information about the role of ADAMTS4 in diabetes. But, based on the active role of ADAMTS4 in degradation of the extracellular matrix and atherogenesis, we hypothesize that ADAMTS4 may be responsible for diabetes-associated atherosclerosis.

There was no difference TGFβ1 serum levels between diabetics with non-diabetics in our study. Higher TGFβ1 serum levels were shown in diabetics, correlating with serum glucose levels (37). This condition may be the result of diabetic patients being under treatment and normal serum glucose levels. The effect of increased TGFβ1 levels in diabetics on atherogenesis is not known yet. Further clinical studies are needed for the role of ADAMTS4 and the association with TGFβ1 and serum glucose levels in diabetes.

Study limitations

On the tissue level, we did not show the expression level of TGFβ1 and ADAMTS4 in the vascular wall. Tissue-specific expression investigations must be planned for confirmation of the association between ADAMTS4 and TGFβ1 in the vascular wall.

We hypothesized TGFβ1 signaling pathway defects may be responsible for the progression of disease, but our study did not demonstrate TGFβ1 signaling defects. In vitro cell culture investigation from patient monocyte-macrophages must be planned for the signaling defect.

In diabetics, we could not explain the etiology of higher ADAMTS4 levels and the association with diabetics' clinical features and TGFβ1 serum levels.

For TGFβ1 haplotypes, the sample size is small, and the genetic susceptibility for CAD must be confirmed with larger sample sizes.

828 Uluçay et al.
TGFβ1 and ADAMTS4 in coronary disease

Anatol J Cardiol 2015; 15: 823-9

Conclusion

The present study demonstrated that ADAMTS4 may have a critical role in atherogenesis. Determining the secondary signal that regulates ADAMTS4 expression is necessary for preventive treatment in CAD. Our data first showed clinically based findings about the association between TGFβ1 and ADAMTS4 for the progression of atherosclerosis, and the mechanism of disease is associated with atherosclerosis in diabetics. These results suggested that the TGFβ1 signaling pathway and ADAMTS4 have an important role for the progression of atherogenesis.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - F.S.Ç., S.U.; Design - K.D.; Supervision - F.S.Ç., R.S.; Resource - M.B.B.; Materials - Ö.B., M.B.B.; Data collection &/or processing - Ö.B., M.B.B.; Analysis &/or interpretation - R.S., K.D.; Literature search - S.U.; Writing - S.U., F.S.Ç., K.D.; Critical review - R.S., Ö.B., F.S.Ç., K.D., M.B.B., S.U.

References

1. Stocker R, Keaney JF Jr. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004; 84: 1381-478. **[CrossRef]**
2. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 1995; 15: 551-61. **[CrossRef]**
3. Williams KJ. Arterial wall chondroitin sulfate proteoglycans: diverse molecules with distinct roles in lipoprotein retention and atherogenesis. Curr Opin Lipidol 2001; 12: 477-87. **[CrossRef]**
4. Wight TN. Versican: a versatile extracellular matrix proteoglycan in cell biology. Curr Opin Cell Biol 2002; 14: 617-23. **[CrossRef]**
5. Kenagy RD, Plaas AH, Wight TN. Versican degradation and vascular disease. Trends Cardiovasc Med 2006; 16: 209-15. **[CrossRef]**
6. Sandy JD, Westling J, Kenagy RD, Iruela-Arispe ML, Verscharen C, Rodriguez-Mazaneque JC, et al. Versican V1 proteolysis in human aorta in vivo occurs at the Glu441-Ala442 bond, a site that is cleaved by recombinant ADAMTS-1 and ADAMTS-4. J Biol Chem 2001; 276: 13372-8. **[CrossRef]**
7. Salter RC, Ashlin TG, Kwan AP, Ramji DP. ADAMTS proteases: key roles in atherosclerosis? J Mol Med 2010; 88: 1203-11. **[CrossRef]**
8. Chen L, Yang L, Zha Y, Cui L. Association of serum a disintegrin and metalloproteinase with thrombospodin motif 4 levels with the presence and severity of coronary artery disease. Coron Artery Dis 2011; 22: 570-6. **[CrossRef]**
9. Zha Y, Chen Y, Xu F, Li T, Zhao C, Cui L. ADAMTS4 level in patients with stable coronary artery disease and acute coronary syndromes. Biomed Pharmacother 2010; 64: 160-4. **[CrossRef]**
10. Wågsäter D, Björk H, Zhu C, Björkegren J, Valen G, Hamsten A, et al. ADAMTS-4 and -8 are inflammatory regulated enzymes expressed in macrophage-rich area of human atherosclerotic plaques. Atherosclerosis 2008; 196: 514-22. **[CrossRef]**
11. Salter RC, Arnaoutakis K, Michael DR, Singh NN, Ashlin TG, Buckley ML, et al. The expression of a disintegrin and metalloproteinase with thrombospondin motifs 4 in human macrophages is inhibited by the anti-atherogenic cytokine transforming growth factor-β and requires Smads, p38 mitogen-activated protein kinase and c-Jun. Int J Biochem Cell Biol 2011; 43: 805-11. **[CrossRef]**
12. Grainger DJ. Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. Arterioscler Thromb Vasc Biol 2004; 24: 399-404. **[CrossRef]**
13. Khan R, Agrotis A, Bobik A. Understanding the role of transforming growth factor-beta1 in intimal thickening after vascular injury. Cardiovasc Res 2007; 74: 223-34. **[CrossRef]**
14. Ghosh J, Murphy MO, Turner N, Khwaja N, Halka A, Kielty CM, et al. The role of transforming growth factor beta1 in the vascular system. Cardiovasc Pathol 2005; 14: 28-36. **[CrossRef]**
15. Gamble JR, Khew-Goodall Y, Vadas MA. Transforming growth factor-beta inhibits E-selectin expression on human endothelial cells. J Immunol 1993; 150: 4494-503.
16. Fihn SD, Gardin JM, Abrams J, Berra K, Blankenship JC, Dallas AP, et al. 2012 ACCF/AHA/ACP/AATS/PCNA/SCAI/STS Guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology Foundation/ American Heart Association Task Force on Practice Guidelines, and the American College of Physicians, American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Am Coll Cardiol 2012; 60: e44-e164. **[CrossRef]**
17. Newby AC. Dual role of matrix metalloproteinases (matrixins) in intimal thickening and atherosclerotic plaque rupture. Physiol Rev 2005; 85: 1-31. **[CrossRef]**
18. Tortorella MD, Malfait F, Barve RA, Shieh HS, Malfait AM. A review of the ADAMTS family, pharmaceutical targets of the future. Curr Pharm Des 2009; 15: 2359-74. **[CrossRef]**
19. Chen YC, Bui AV, Diesch J, Manasseh R, Hausding C, Rivera J, et al. A novel mouse model of atherosclerotic plaque instability for drug testing and mechanistic/therapeutic discoveries using gene and microRNA expression profiling. Circ Res 2013; 113: 252-65. **[CrossRef]**
20. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 1999; 8: 93-7. **[CrossRef]**
21. Shah R, Rahaman B, Hurley CK, Posch PE. Allelic diversity in the TGFB1 regulatory region: characterization of novel functional single nucleotide polymorphisms. Hum Genet 2006; 119: 61-74. **[CrossRef]**
22. Crobu F, Palumbo L, Franco E, Bergerone S, Carturan S, Guarrera S, et al. Role of TGF-β1 haplotypes in the occurrence of myocardial infarction in young Italian patients. BMC Med Genet 2008; 9: 13. **[CrossRef]**
23. Yokota M, Ichihara S, Lin TL, Nakashima N, Yamada Y. Association of a T29-->C polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to myocardial infarction in Japanese. Circulation 2000; 101: 2783-7. **[CrossRef]**
24. Koch W, Hoppmann P, Mueller JC, Schömig A, Kastrati A. Association of transforming growth factor-beta1 gene polymorphisms with myocardial infarction in patients with angiographically proven coronary heart disease. Arterioscler Thromb Vasc Biol 2006; 26: 1114-9. **[CrossRef]**

Anatol J Cardiol 2015; 15: 823-9

Uluçay et al.
TGFβ1 and ADAMTS4 in coronary disease **829**

25. Syrris P, Carter ND, Metcalfe JC, Kemp PR, Grainger DJ, Kaski JC, et al. Transforming growth factor-beta1 gene polymorphisms and coronary artery disease. Clin Sci 1998; 95: 659-67. **[CrossRef]**

26. Deng HB, Jiang CQ, Tomlinson B, Liu B, Lin JM, Wong KS, et al. A polymorphism in transforming growth factor-β1 is associated with carotid plaques and increased carotid intima-media thickness in older Chinese men: the Guangzhou Biobank Cohort Study-Cardiovascular Disease Subcohort. Atherosclerosis 2011; 214: 391-6. **[CrossRef]**

27. Tashiro H, Shimokawa H, Yamamoto K, Momohara M, Tada H, Takeshita A. Altered plasma levels of cytokines in patients with ischemic heart disease. Coron Artery Dis 1997; 8: 143-7. **[CrossRef]**

28. Lu Y, Boer JM, Barsova RM, Favorova O, Goel A, Müller M, et al; PROCARDIS CARDIoGRAM Consortium. TGFB1 genetic polymorphisms and coronary heart disease risk: a meta-analysis. BMC Med Genet 2012; 13: 39. **[CrossRef]**

29. Grainger DJ, Kemp PR, Metcalfe JC, Liu AC, Lawn RM, Williams NR, et al. The serum concentration of active transforming growth factor-beta is severely depressed in advanced atherosclerosis. Nat Med 1995; 1: 74-9. **[CrossRef]**

30. Chen C, Lei W, Chen W, Zhong J, Gao X, Li B, et al. Serum TGF-β1 and SMAD3 levels are closely associated with coronary artery disease. BMC Cardiovasc Disord 2014; 14: 18. **[CrossRef]**

31. Singh NN, Ramji DP. The role of transforming growth factor-beta in atherosclerosis. Cytokine Growth Factor Rev 2006; 17: 487-99. **[CrossRef]**

32. Toma I, McCaffrey TA. Transforming growth factor-β and atherosclerosis: interwoven atherogenic and atheroprotective aspects. Cell Tissue Res 2012; 347: 155-75. **[CrossRef]**

33. Rook GA. The TGF-beta1 paradox in asthma. Trends Immunol 2001; 22: 299-300. **[CrossRef]**

34. Redondo S, Navarro-Dorado J, Ramajo M, Medina Ú, Molina-Sanchez P, Garces Z, et al. Age-dependent defective TGF-beta1 signaling in patients undergoing coronary artery bypass grafting. J Cardiothorac Surg 2014; 9: 24. **[CrossRef]**

35. Pham Y, Tu Y, Wu T, Allen TJ, Calkin AC, Watson AM, et al. Cell division autoantigen 1 plays a profibrotic role by modulating downstream signalling of TGF-beta in a murine diabetic model of atherosclerosis. Diabetologia 2010; 53: 170-9. **[CrossRef]**

36. Ho MM, Yoganathan P, Chu KY, Karunakaran S, Johnson JD, Clee SM. Diabetes genes identified by genome-wide association studies are regulated in mice by nutritional factors in metabolically relevant tissues and by glucose concentrations in islets. BMC Genet 2013; 14: 10. **[CrossRef]**

37. Yang SN, Burch ML, Tannock LR, Evanko S, Osman N, Little PJ. Transforming growth factor-β regulation of proteoglycan synthesis in vascular smooth muscle: contribution to lipid binding and accelerated atherosclerosis in diabetes. J Diabetes 2010; 2: 233-42. **[CrossRef]**