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RESEARCH ARTICLE

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Genetic variants rs1994016 and rs3825807 in ADAMTS7 affect its mRNA expression in atherosclerotic occlusive peripheral arterial disease

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Aim: Peripheral artery disease (PAD) is a vascular disease affecting peripheral circulation. Recently, genome-wide association studies revealed a relationship between single nucleotide polymorphisms (SNPs) in *ADAMTS7* (a disintegrin and metalloprotease with thrombospondin motif 7) and atherosclerosis. In this study, we aimed to determine ADAMTS7 expression in peripheral blood mononuclear cells (PBMCs) and the frequency of *ADAMTS7* rs1994016 and rs3825807 polymorphisms in a sample of Turkish patients with PAD, and to evaluate the association of matrix metalloproteinase (MMP) levels with PAD development.

Methods: In this case–control study, *ADAMTS7* mRNA and protein expression was determined using reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) and western blot, respectively, and rs1994016 and rs3825807 variants in *ADAMTS7* were determined by real-time PCR in 115 PAD patients and 116 healthy controls. Plasma levels of nine MMPs were determined using a multiplex immunoassay system.

Results: *ADAMTS7* mRNA levels were significantly higher in PAD patients than in controls (t=−2.75, P=.007). There was no significant difference in the frequencies of rs1994016 and rs3825807 between PAD patients and controls (P>.05). In PAD patients, *ADAMTS7* mRNA levels were significantly increased for the CC genotype of rs1994016 (t=−2.31, P=.026) and TT genotype of rs3825807 (t=−2.23, P=.032). Furthermore, plasma levels of MMP-1, MMP-3, MMP-7, MMP-10, MMP-12, and MMP-13 were significantly higher in PAD patients than in controls (P<.05).

Conclusion: This is the first report of the relationship between PAD and ADAMTS7 expression and the effects of the rs1994016 and rs3825807 variants on PAD development. ADAMTS7 may be associated with PAD development.

KEYWORDS
ADAMTS7, gene expression, matrix metalloproteinases, peripheral artery disease

1 | INTRODUCTION

Peripheral artery disease (PAD) is a type of occlusive disease that affects the peripheral circulation, and atherosclerosis is the most common cause of PAD. Atherosclerosis is characterized by plaques formed from inflammatory cells, lipid deposition, fibrosis, calcification, necrotic cells, smooth muscle cell (SMC) proliferation and necrosis, and disruption of the elastic lamina. The prevalence of PAD is quite high, affecting more than 200 million people worldwide.[1] The main presentations of PAD include claudication or critical limb ischemia and abnormal ankle

brachial index (ABI).[2] Both genetic and environmental risk factors play important roles in PAD development. Hypertension, hyperlipidemia, diabetes mellitus, abnormal homocysteine levels, obesity, cigarette smoking, and familial predisposition are some of the risk factors. PAD development may also be independently influenced by genetic factors.

Recently, genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) in *ADAMTS7* (a disintegrin and metalloprotease with thrombospondin motif 7), and these SNPs were associated with coronary artery disease (CAD) in several populations.[3-6] ADAMTS7 belongs to the ADAMTS family of disintegrins and metalloproteases with thrombospondin type-1 repeats, and it contains a signal peptide, a prodomain, a disintegrin-like domain, and a thrombospondin type-1 motif. The prodomain is cleaved during ADAMTS7 maturation and activation.[7] Activated ADAMTS7 has proteolytic activity, and its best-known substrate is thrombospondin-5 (TSP 5; also known as COMP), which is an extracellular matrix (ECM) protein found in vascular walls and cartilage.[8,9] Studies have shown that ADAMTS7 is involved in the degradation of TSP 5, which initiates vascular smooth muscle cell (VSMC) migration following vascular injury.[9]

Various proteinases are produced in atherosclerosis. Inflammatory macrophages and foam cells trigger the destruction of structural ECM components, especially collagens, and overstimulation of resident VSMCs and endothelial cells that provide tensile strength.[10] A study demonstrated that siRNA-mediated inhibition of *ADAMTS7* reduced the proteolysis of TSP 5 and impaired VSMC migration in culture and intima formation in a rat carotid balloon injury model.[9]

Atherosclerosis is an inflammatory process involving matrix metalloproteinase (MMP) activation. MMPs degrade collagen and allow SMC migration within a vessel, which also leads to the accumulation of cellular materials, resulting in vessel occlusion and ischemia.[10] It was previously reported that activation of the MMP-8/ADAM-10 cascade increased the proliferation and migration of mouse VSMCs and promoted neointima formation.[11]

Polymorphisms and mRNA expression of the ADAMTS7 gene and related MMP levels may have a phenotypic and clinical significance in PAD development. Exploring the possible relationship between the ADAMTS7 gene and PAD may contribute to elucidation of the molecular pathogenesis of PAD and may be useful in future treatment strategies. To date, the association of *ADAMTS7* mRNA and protein levels and the rs1994016 and rs3825807 (Ser214Pro) polymorphisms with PAD and with plasma MMP levels has not been reported. Thus, we tested the hypothesis that *ADAMTS7* levels and the rs1994016 and rs3825807 variants play a role in PAD development and investigated whether *ADAMTS7* mRNA and protein levels, rs1994016 and rs3825807 polymorphisms, and plasma MMP levels are associated with PAD and its clinical parameters.

2 | MATERIALS AND METHODS

2.1 | Subjects

This study included 231 Turkish subjects consisting of 115 patients with PAD (90 male, 25 female; mean age 61.24±11.06 years; range 36-82 years) and 116 healthy controls (87 male, 29 female; mean age 60.15±8.04 years; range 40-82 years) selected from the Istanbul University Cerrahpasa Medical Faculty, Cardiovascular Surgery Department. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Local Ethics Committee of the Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. Informed consent was obtained from all individual participants included in the study.

Eligible patients were recruited on the basis of atherosclerotic stenosis of the peripheral arteries (≥50%) detected by physical examination, ankle brachial index (ABI), duplex Doppler ultrasound, computed tomography-angiography, magnetic resonance angiography, and digital subtraction angiography. The recruited PAD patients all underwent surgery at our department, which took place after the blood tests, and all were actively using statins.

The control group consisted of 116 randomly selected healthy individuals who visited our hospital for regular health screenings without any clinical findings of PAD. The healthy controls were not included in the study if intermittent claudication with palpable pulses in the arteries of the lower extremities were identified upon examination. Additional inclusion criteria were normal lipid profile and no statin use. Exclusion criteria were presence of cardiovascular disease, diabetes, hypertension, any atherosclerosis risk factor, autoimmune disease, severe kidney and hepatic diseases, cancer, and pregnancy. All patients and controls were Turkish, from the same geographical area, and with similar socioeconomic backgrounds (middle-income status) as assessed from patients' responses to a health questionnaire.

2.2 | Blood samples and DNA isolation

Venous blood samples from all participants were collected into EDTA tubes for DNA isolation and stored in aliquots at −20°C until use. Genomic DNA was extracted from whole blood using a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

2.3 | Blood sampling, peripheral blood mononuclear cell (PBMC) isolation, and RNA isolation

Venous blood samples obtained from all participants were collected into heparin tubes and immediately used for lymphocyte separation and RNA extraction. PBMCs were isolated from 10 mL of sodium heparin-treated venous blood samples by Ficoll-Hypaque gradient centrifugation. Total RNA was extracted from freshly isolated PBMCs by using the PureLink RNA Mini Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.4 | Reverse transcription quantitative real-time PCR (RT-qPCR)

Total RNA (400 ng) was reverse transcribed using random hexamers as primers and reverse transcriptase, with a total volume of 20 μL. *ADAMTS7* expression in PBMCs was determined by quantitative

real-time polymerase chain reaction (RT-qPCR) by using a LightCycler 1.5 system (Roche Applied Biosystems, CA, USA) with UPL probes and the TaqMan method. *ADAMTS7* mRNA levels were normalized to *ACTB*, *B2M*, and *GAPDH* mRNA levels, which were used as endogenous reference genes. The following primer sequences were used: *ADAMTS7*, 5′-AGC CGC CAG TAT ATC ACC AG-3′ (forward) and 5′- GAG GGG AAG TCG ATA ATG TCC -3′ (reverse); *ACTB*, 5′-AGA GCT ACG AGC TGC CTG AC-3′ (forward) and 5′-CGT GGA TGC CAC AGG ACT-3′ (reverse); and *B2M*, 5′-ATC TGA GCA GGT TGC TCC AC-3′ (forward) and 5′-GAC CAA GAT GTT GAT GTT GGA TAA-3′ (reverse); *GAPDH*, 5′- AGC CAC ATC GCT CAG ACA C -3′ (forward) and 5′- GCC CAA TAC GAC CAA ATC C-3′ (reverse). Reaction mixtures without a cDNA template were utilized as negative controls. Ct values of 40 were excluded from the study. The experiments were performed twice.

2.5 | Genotyping of rs1994016 and rs3825807 SNPs in the ADAMTS7 gene

The rs1994016 and rs3825807 SNPs in *ADAMTS7* were determined using the LightCycler 1.5 system with hybridization probes (TIB MOLBIOL GmbH, Berlin, Germany). Genotyping was performed in a 20-μL volume containing 2.0 μL of DNA Master mix (Roche Diagnostics GmbH), 1.0 μL of Reagent Mix, 3.0 mmol/L $MgCl_2$, and 50 ng of genomic DNA. The quality of the SNP genotyping was verified by independently replicating the genotyping by using randomly selected samples; the quality control results were in agreement with the initial genotyping results.

2.6 | Multiplex immunoassay

Levels of plasma MMPs, that is, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-12, and MMP-13, were measured using a Bio-Plex multiplex immunoassay (Bio-Rad, Hercules, CA, USA) system according to the manufacturer's instructions. Plates were BSA-blocked and washed. MMP standards, controls, and unknown samples were added (50 μL total volume) to 96-well plates and incubated with antibody-immobilized microbeads. After washing, biotinylated detection antibodies were incubated with the bound MMPs. Fluorescent (phycoerythrin-labeled) streptavidin was added. A final wash was followed by resuspension in sheath fluid for analysis in the Bio-Plex array reader (Bio-Rad) using Bio-Plex Manager 4.1 software. A standard curve was constructed using a 5-parameter logistic (5PL) regression, and the concentration of each MMP (pg/mL) was calculated against this curve.

2.7 | Western blot analysis

Peripheral blood mononuclear cell extracts were collected and the total protein was isolated using a Total Protein Extraction Kit (Chemicon International, Billerica, MA, USA). Total proteins were separated by SDS gel electrophoresis and transferred onto PVDF membranes, which were incubated with rabbit anti-human ADAMTS7 polyclonal antibody (1:3000 dilution; Abcam, Cambridge, MA, USA), rabbit anti-human ACTB polyclonal antibody (1:5000 dilution; Abcam), and goat anti-rabbit IgG peroxidase-conjugated H+L antibody (1:5000 dilution; Abcam). Specific bands were detected using a Bio-Rad imaging system (Bio-Rad). Relative protein expression change was determined using Image J software (free software from National Institutes of Health, Bethesda, MD, USA).

2.8 | Statistical analysis

Continuous variables were compared between PAD patients and healthy controls by using Student's *t* test; data are expressed as the mean±standard deviation (SD). The chi-squared (χ^2) test was used for categorical variables, including genotype and allele frequencies to compare the association between genotypes and alleles among cases and controls, and to test the deviation of genotype distribution from Hardy–Weinberg equilibrium (HWE). The threshold for statistical significance was $P \leq .05$. Odds ratios (OR) and 95% CI of SNP and gene expression levels were estimated by multiple logistic regression analysis with adjustment for total and LDL cholesterol levels. Relative mRNA levels were evaluated using the $2^{-\Delta Ct}$ method. Results were analyzed by Student's ***t***-test, and data are presented as the mean±standard deviation (SD). Relative gene expression levels and biochemical parameters were also compared using Spearman's non-parametric correlation test. All statistical analyses were performed using SPSS software for Windows (Version 21.0) (IBM Corp, Armonk, NY, USA).

3 | RESULTS

3.1 | Patient demographics

There were no statistically significant differences between the groups with respect to age ($P=.39$) or gender ($P=.33$). However, PAD patients and controls demonstrated significant differences in hematocrit, fasting glucose, urea, creatinine, serum CRP levels ($P<.001$), and systolic blood pressure ($P=.01$), but not in AST, ALT, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride, or diastolic blood pressure levels ($P>.05$; Table 1).

3.2 | ADAMTS7 mRNA and protein levels

ADAMTS7 mRNA levels were significantly increased in PAD patients compared to healthy controls ($t=-2.75$, $P=.007$; Figure 1). ADAMTS7 protein levels were also demonstrated to be higher in PAD patients than in controls (Figure 2).

3.3 | Genotype frequencies of ADAMTS7 rs1994016 and rs3825807 SNPs

The genotype distributions for the rs1994016 and rs3825807 SNPs in PAD patients and controls are presented in Table 2; these distributions were consistent with HWE expectations among patients ($\chi^2=0.81$, $P=.36$; $\chi^2=1.44$, $P=.22$, respectively) and controls ($\chi^2=0.21$, $P=.64$; $\chi^2=0.01$, $P=.88$ respectively).

TABLE 1 Demographic and clinical characteristics of PAD patients and controls

Characteristics	PAD group (n=115)	Control group (n=116)	*P*
Age (y, mean±SD)	61.24±11.06	60.15±8.04	.39
Gender (M/F, %)	90/25 (78.3/21.7)	87/29 (75.0/25.0)	.33
Hematocrit (%)	36.68±6.49	40.60±4.51	<.001
Fasting glucose (mg/dL)	137.13±69.47	96.00±32.19	<.001
AST (U/L)	22.07±13.71	19.60±7.13	.106
ALT (U/L)	20.84±15.16	21.39±9.25	.756
T-cholesterol (mg/dL)	188.39±40.67	193.87±33.92	.337
HDL-cholesterol (mg/dL)	43.22±14.36	47.80±18.01	.063
LDL-cholesterol (mg/dL)	125.10±78.37	130.57±33.20	.571
Triglycerides (mg/dL)	163.03±174.00	166.15±66.84	.889
Urea (mg/dL)	44.80±25.80	33.67±10.35	<.001
Creatinine (mg/dL)	1.227±1.169	0.802±0.222	<.001
C-Reactive Protein (nmol/L)	39.71±60.17	4.03±4.22	<.001
Systolic blood pressure (mm Hg)	128.22±11.53	132.03±10.77	.010
Diastolic blood pressure (mm Hg)	79.48±10.45	77.80±11.74	.253

Student's *t*-test and χ^2 test were performed. Data presented as mean±SD.

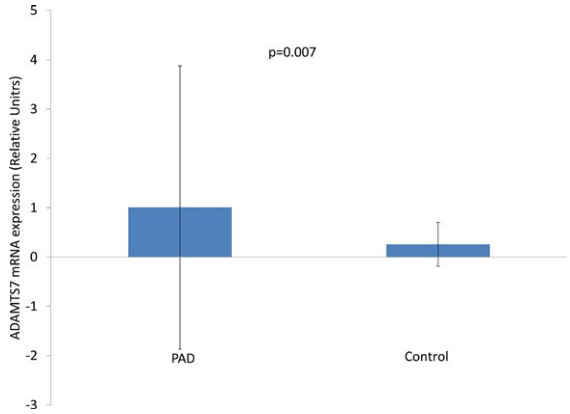

FIGURE 1 Relative mRNA expression of *ADAMTS7* in PAD patients and healthy controls

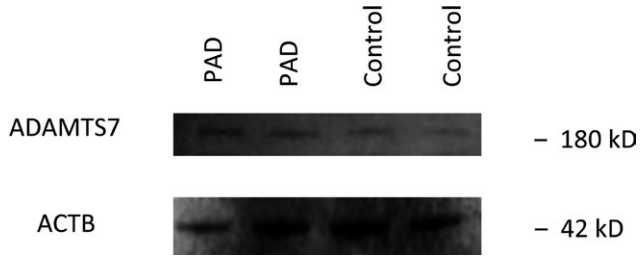

FIGURE 2 ADAMTS7 protein expression levels in PAD cases and controls

No significant difference was observed in the genotype frequencies of the *ADAMTS7* rs1994016 SNP between PAD patients and controls (*P*=.185). However, the rs1994016 T allele frequency was higher in PAD patients than in controls, although the difference was not significant (*P*=.06). There was also no significant difference between genotypic frequencies for the rs3825807 SNP (*P*=.177; Table 2).

3.4 | ADAMTS7 gene expression with regard to rs1994016 and rs3825807 genotypes

ADAMTS7 mRNA levels were significantly increased for the CC genotype of SNP rs1994016 (*t*=−2.31, *P*=.026), and the TT genotype of SNP rs3825807 in PAD patients (*t*=−2.23, *P*=.032; Figure 3).

3.5 | Plasma MMP levels

Plasma MMP-1, MMP-3, MMP-7, MMP-10, MMP-12, and MMP-13 levels were significantly higher in PAD patients than in controls (*P*<.05; Figure 4).

3.6 | Correlation of ADAMTS7 gene expression with triglyceride and LDL and HDL cholesterol levels

In PAD patients, plasma MMP-8 levels were significantly correlated with triglyceride (*r*=.457, *P*=.015), total cholesterol (*r*=.40, *P*=.029), and LDL cholesterol levels (*r*=.462, *P*=.009). Furthermore, plasma MMP-9 levels were significantly correlated with LDL cholesterol levels (*r*=.377, *P*=.037; Figure 5), and plasma MMP-10 levels were significantly correlated with triglyceride (*r*=.441, *P*=.019), total cholesterol (*r*=.482, *P*=.007), and LDL-cholesterol levels (*r*=.515, *P*=.003) in PAD patients. MMP-13 levels were also significantly correlated with triglyceride (*r*=.521, *P*=.004), total cholesterol (*r*=.538, *P*=.002), and LDL cholesterol levels (*r*=.538, *P*=.002) in PAD patients.

3.7 | ADAMTS7 gene expression and ADAMTS7 rs1994016 and rs3825807 SNPs with regard to PAD risk factors

We analyzed the potential selective effects of *ADAMTS7* mRNA levels and *ADAMTS7* rs1994016 and rs3825807 SNPs in patients by using

TABLE 2 Distribution of rs1994016 and rs3825807 genotypes and allele frequencies among PAD patients and controls

Genotype/allele	PAD patients n (%)	Controls n (%)	*P*
ADAMTS7 rs1994016			
CC	38 (33.0)	49 (42.2)	
CT	52 (45.2)	51 (44.0)	
TT	25 (21.7)	16 (13.8)	.185
C allele frequency	0.56	0.64	
T allele frequency	0.44	0.36	.06
ADAMTS7 rs3825807			
TT	34 (29.6)	42 (36.2)	
TC	51 (44.3)	55 (47.4)	
CC	30 (26.1)	19 (16.4)	.177
T allele frequency	0.52	0.60	
C allele frequency	0.48	0.40	.07

χ^2 test was performed. Data are presented as n (%).

multiple logistic regression analysis with adjustments for certain PAD risk factors. Risk factors including serum CRP levels (*P*=.038, OR=1.272; 95% CI: 1.014-1.597) together with high *ADAMTS7* mRNA levels were significantly related to PAD development. Moreover, the presence of hypertension was associated with PAD pathogenesis with borderline significance (*P*=.056, OR=0.109; 95% CI: 0.011-1.059; Table 3).

4 | DISCUSSION

Several GWAS have revealed that SNPs in *ADAMTS7* are associated with CAD.[3-5] The CAD-related SNP rs3825807 results in an amino acid substitution (Ser-to-Pro) in the ADAMTS7 prodomain.[5] The effects of two other SNPs, rs1994016 in intron 8[4] and rs4380028 at 7.6 kb upstream of the gene,[3] on *ADAMTS7* expression or activity in CAD development remain unclear. However, to the best of our knowledge, the association between ADAMTS7 levels, rs1994016 and rs3825807, and plasma MMP levels in PAD pathogenesis has not been reported previously.

SiRNA-mediated inhibition of *ADAMTS7* was shown to reduce the proteolysis of TSP 5 and impaired VSMC migration in culture.[9] It has also been reported that ADAMTS7 promotes VSMC proliferation both in vitro and in vivo and may therefore serve as a therapeutic target for atherosclerosis.[12] TSP 5, which is produced by VSMCs and which inhibits VSMC migration, has also been demonstrated to suppress VSMC phenotypic modulation, thereby promoting neointima formation.[13,14] Because VSMC migration is a significant component of atherogenesis, *ADAMTS7* may play a role in PAD development as well. However, the underlying mechanism is not yet understood. In our study, PAD patients and controls demonstrated significant differences in the levels of hematocrit, fasting glucose, urea, creatinine, serum CRP, and systolic blood pressure but not in the levels of AST; ALT; total, HDL, and LDL cholesterol; diastolic blood pressure; and triglycerides. Furthermore, *ADAMTS7* mRNA levels were significantly higher in PAD patients than in healthy controls, suggesting that *ADAMTS7* may play a significant role in peripheral circulation and its pathologies such as PAD.

A GWAS reported that lower susceptibility to CAD was associated with rs3825807.[5] Another GWAS found that the rs1994016 variant was associated with the incidence of symptomatic CHD but not with that of MI.[4] However, none of these studies clarified whether the SNPs have functional effects on *ADAMTS7* expression or activity in CAD development. In the present study, no significant difference was

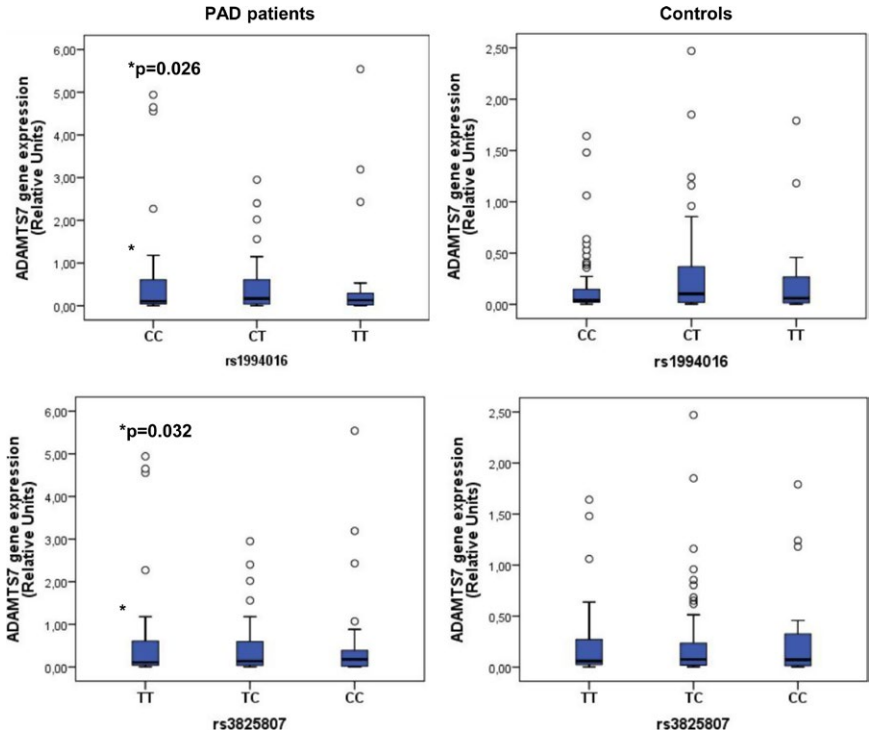

FIGURE 3 *ADAMTS* mRNA expression with regard to rs1994016 and rs3825807 variants

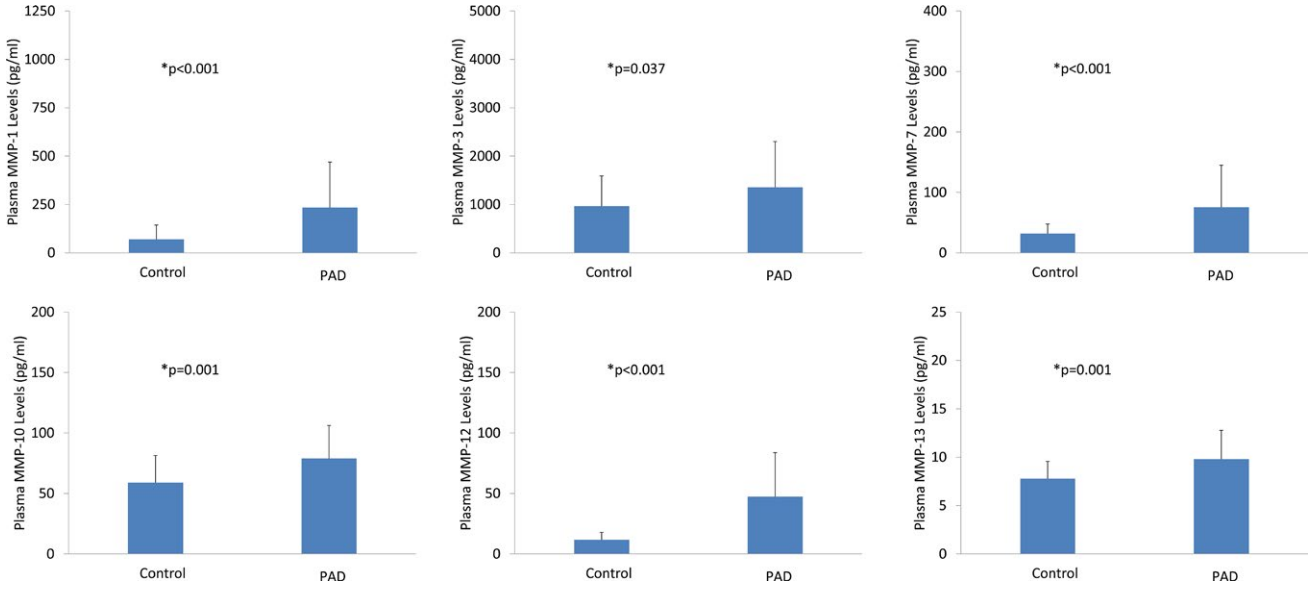

FIGURE 4 Plasma MMP levels among PAD patients and controls

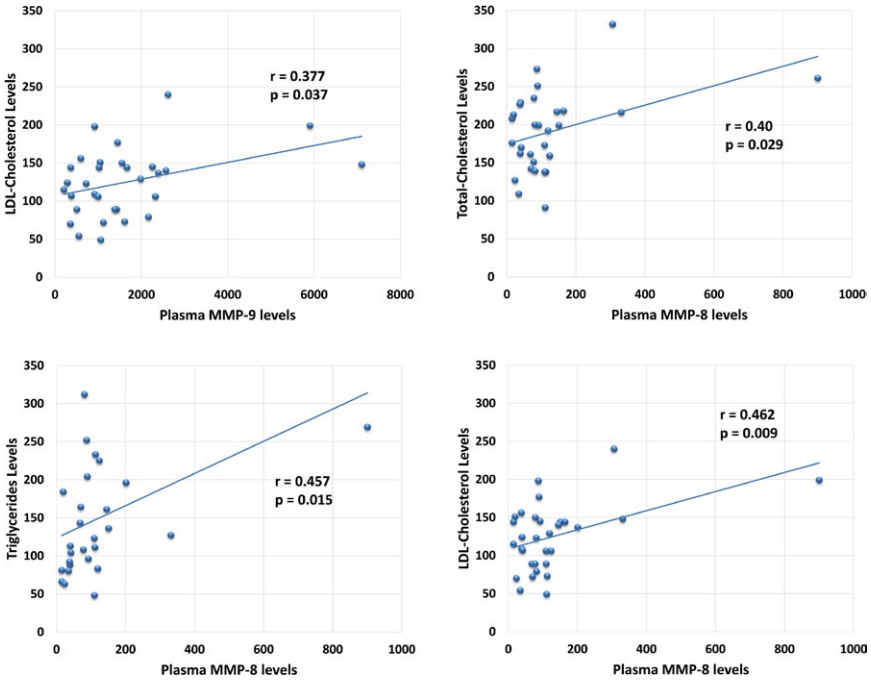

FIGURE 5 Correlation of MMP-8 and -9 levels with blood lipid parameters

observed in the genotype frequencies of rs1994016 between PAD patients and controls. However, the T allele frequency of rs1994016 was higher in PAD patients than in controls, although the difference was not significant. We also did not observe any significant difference in the genotype frequencies of rs3825807 between PAD patients and controls. This result may be due to the small sample size. However, the risk alleles rs1994016 (C allele) and rs3825807 (T allele) were found to influence PAD pathogenesis in our study. Furthermore, considering the localization of the SNPs in relation to *ADAMTS7*, they may influence *ADAMTS7* expression.

Pu et al.[15] reported that rs3825807 had a suppressive effect on ADAMTS7 maturation, TSP 5 cleavage, and VSMC migration. They also reported that Ser-to-Pro substitution reduced ADAMTS7 prodomain processing and that the *ADAMTS7* rs3825807 genotype (Pro) was associated with lower atherosclerosis prevalence and severity. An association between *ADAMTS7* rs3825807 and aortic calcification has also been reported.[16] You et al.[17] reported that *ADAMTS7* rs3825807 was associated with CAD susceptibility and severity in a Chinese population. Consistent with these reports, in our study, *ADAMTS7* expression levels were significantly higher for the TT genotype of rs3825807 among PAD patients. In the rs3825807 Ser214Pro polymorphism, the amino acid change from serine (polar) to proline (non-polar) may influence ADAMTS7 levels because this change occurs in the prodomain of the protein. Furthermore, in our study, *ADAMTS7* mRNA levels were significantly increased for the

TABLE 3 Associations between ADAMTS7 mRNA expression and some PAD risk factors

	P value	Exp (B)	95.0% CI	
			Lower	Upper
ADAMTS7 mRNA expression	.24			
Serum CRP levels	.038	1.272	1.014	1.597
Hypertension (+)	.056	0.109	0.011	1.059
Constant	.170	4.656		

Adjustments for age, gender, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, total-cholesterol, triglycerides, C-reactive protein, systolic blood pressure, having hypertension and diabetes, *ADAMTS7* mRNA expression and rs1994016 and rs3825807 variants were performed.
Exp (B), exponentiation of the B coefficient; 95% CI, difference of means at the 95% confidence interval.

CC genotype of rs1994016 among PAD patients, suggesting a potential allelic effect on ADAMTS7 expression in PAD development.

Matrix metalloproteinases have been reported to exert both adverse and protective effects on plaque rupture in animal models.[18] MMPs may be involved in VSMC proliferation via indirect effects, such as release of matrix-anchored growth factors or removal of cell-cell contact proteins.[19] However, metalloproteinases other than MMPs may contribute to enhancement of VSMC proliferation in vivo. ADAMTS7 enhances VSMC proliferation both in vitro and in vivo.[12] Wu et al.[20] investigated plasma ADAMTS7 levels in acute myocardial infarction (AMI) and reported that ADAMTS7 levels may be associated with heart failure after AMI.

In atherosclerosis, high production of a wide range of proteinases triggers the recruitment of inflammatory macrophages and foam cells and overstimulates resident VSMCs and endothelial cells, which leads to destruction of ECM components.[10] In our study, plasma MMP-1, MMP-3, MMP-7, MMP-10, MMP-12, and MMP-13 levels were significantly higher in PAD patients than in controls. Furthermore, in PAD patients, plasma MMP-8, MMP-10, and MMP-13 levels were significantly correlated with the levels of triglycerides and total and LDL cholesterol, whereas plasma MMP-9 levels were significantly correlated with LDL cholesterol levels. Moreover, risk factors such as serum CRP levels and *ADAMTS7* expression were significantly associated with PAD development. Based on this evidence, we suggest that ADAMTS7 and various MMPs may play a role in the inflammatory mechanisms in PAD pathogenesis.

5 | CONCLUSION

Because PAD is a multifactorial and complex disease, its molecular pathogenesis is still unknown. Our study has some limitations; a larger sample size and analysis of serum/plasma ADAMTS7 protein levels to confirm the results would also help us to understand the disease pathogenesis. However, although our study has some limitations, it is the first study to evaluate the possible role of the ADAMTS7 gene and various MMPs in PAD development, and our findings suggest a possible relationship between MMP-related genes and atherosclerotic PAD. The results of our study suggest that the ADAMTS7 gene may be associated with PAD development in this study population and that the rs1994016 CC and rs3825807 TT genotypes may upregulate ADAMTS7 mRNA levels and may influence PAD development. ADAMTS7 may also have clinical significance in PAD development. Confirmation of these findings in future studies may support the use of ADAMTS7 inhibitors to reduce the severity of atherosclerosis and PAD-related symptoms.

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