ORIGINAL RESEARCH

Involvement of Matrix Metalloproteinase 9 in Vertebral Arterial Dissection With Posterior Circulation Ischemic Stroke

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BACKGROUND: Spontaneous vertebral arterial dissection (VAD) is an important cause of posterior circulation ischemic stroke (PCS), but its pathogenesis remains elusive. Matrix metalloproteinase 9 (MMP-9) is a gelatinase involved in inflammation process and several vascular diseases, such as aorta dissection, but its role in VBD is unclear yet. The present study aimed to determine the association between serum MMP-9 level and VAD-related PCS.

METHODS AND RESULTS: We recruited 149 patients with PCS, of which 30 were VAD and 119 had other determined etiologies (non-VAD), and 219 non-stroke individuals. Serum MMP-9 was measured within 14 days from stroke onset. The age of VAD group was 59.6±15.0 years, which is similar to non-stroke group (P=0.510) but significantly younger than non-VAD group (69.9±14.0 years, P<0.001). Males and vascular risk factors were significantly more prevalent in VAD and non-VAD groups than non-stroke group (P<0.001). Multivariate logistic regression analysis adjusting potential confounders revealed that every 100 ng/mL of serum MMP-9 level increment significantly predicted VAD (versus non-stroke group: odds ratio (OR), 4.572; 95% CI, 2.240–9.333, P<0.001; versus non-VAD group: OR, 1.819; 95% CI, 1.034–3.200, P=0.038).

CONCLUSIONS: Patients with VAD-related PCS had higher levels of serum MMP-9 at the acute stage of stroke compared with non-stroke individuals and PCS of other causes, supporting the potential involvement of extracellular matrix-degrading protease in the mechanism of VAD, which leads to ischemic events.

Key Words: dissection ■ matrix metalloproteinases ■ posterior circulation ■ stroke ■ vertebrobasilar

Spontaneous vertebral artery dissection (VAD), particularly intracranial VAD, has been increasingly recognized as an important cause of posterior circulation ischemic stroke (PCS).[1–4] The outcome of VAD-PCS could be devastating,[5,6] however, its pathophysiology and optimal management is less understood than the other stroke etiologies. We recently found that VAD-PCS is associated with higher leukocyte and neutrophil counts; the results suggest that inflammation and neutrophil-related pathophysiology might be involved in the mechanism of VAD-PCS.[7]

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that degrade several components of the extracellular matrix (ECM) and mediate the vascular remodeling. Abnormalities of MMPs production and activity have been shown to be involved in several vascular diseases in many previous studies.[8–10] MMP-9, a gelatinase bearing the ability to degrade type IV collagen and elastin, the principal components in the arterial vessel wall, is primarily an inducible enzyme and is involved in inflammatory process.[11–13] Clinical studies have shown an association between elevated serum levels of MMP-9 and aorta dissection.[14,15] Animal studies further demonstrated that acute thoracic aorta dissection could be triggered by MMP-9 released by neutrophils infiltrated in the intima.[16] The present study aimed to explore whether MMP-9, which could be induced by inflammation and released by neutrophil, is associated with VAD-PCS.

Nonstandard Abbreviations and Acronyms

BA	basilar artery
BABO	basilar artery atheromatous branch occlusive disease
LAA	large artery atherosclerotic stenosis/occlusion
MMP	matrix metalloproteinase
NS	non-stroke individuals
PCS	posterior circulation ischemic stroke
TVGH	Taipei Veterans General Hospital
VAD	vertebral artery dissection

We hypothesized that patients with VAD-PCS had a significantly higher serum level of MMP-9. Since there would be a rise in serum levels of MMP9 during the acute phase of ischemic stroke,[17–19] the comparison groups in the present study would include PCS of etiologies other than arterial dissection in addition to non-stroke controls.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

We prospectively recruited 149 consecutive patients with PCS confirmed by magnetic resonance imaging (MRI) who were admitted and registered in Taipei Veterans General Hospital (TVGH) between May of 2015 and April of 2018. Recorded data included demographic and vascular risk factors, stroke severity at admission, and results of laboratory and neuroimaging examinations during hospitalization. Every patient with

acute ischemic stroke in TVGH received brain MRI and time-of-flight MR arteriography (MRA), color-coded duplex ultrasound of cervicocerebral arteries, 24-hour Holter monitor, and echocardiogram for stroke cause evaluation. Patients with suspected arterial dissection would undergo an additional high-resolution MRI and MRA with contrast (3 mm in slice thickness) for evaluation of the vessel walls of relevant arteries. PCS patients with: (1) malignancy, (2) autoimmune diseases, (3) hematological diseases, or (4) undetermined etiology were excluded.

Patients' data were reviewed and consensus was reached for their stroke etiologies by two neurologists (Dr Chung and Chen) and one radiologist (Dr Chang), all of whom are stroke specialists. We used standardized criteria to classify stroke etiology. The criteria for etiology determination are described below:[1,20,21]

1. Basilar Artery Atheromatous Branch Occlusive Disease (BABO). Vascular and cardiac studies showed absence of relevant vascular occlusion/severe stenosis and cardioembolic risks. Basilar artery (BA) showed wall thickening by high resolution or standard MRI T1 (isointense) and/or T2/Fluid attenuated inversion recovery (FLAIR) (hypointense) sequence at the level of cerebral infarct. Clinical and brain imaging studies were compatible with paramedian pontine/midbrain/upper medulla infarct (they were usually basal-brainstem involved and wedge-shaped) caused by several penetrating vessels occlusion.

2. Large Artery Atherosclerotic Stenosis/Occlusion (LAA). Vascular studies showed occlusion or severe stenosis (>50%) of the VAs, BA, or the posterior cerebral arteries (PCAs). There should be multiple or diffusely atherosclerotic changes in the other large arteries. Abrupt cut-off of vessels was considered more likely caused by embolism and was not considered for this category. Cerebral infarcts were in the territory of the large artery stenosis/occlusion larger than that of a single branch artery territory, or in distal fields.

3. Vertebral Artery Dissection (VAD). Vascular studies showed: (1) occlusion or severe stenosis (>50%) of the VA; (2) gradually tapered or/and long-segmental narrowing of the stenotic/occlusive vessel; and (3) intramural hematoma, intimal flap, or double lumen by high-resolution or standard MRI T1 sequence with or without contrast. There was usually vascular dilatation with or without aneurysm at the level of dissection. Associated atherosclerotic changes in the other large arteries were acceptable. Cerebral infarcts were in the territory of the dissected vessel larger than that of a single branch artery territory, or in distal fields (in most cases).

4. Cardioembolism. Vascular studies showed an abrupt cut-off of branch or main artery (usually the distal part), or patency of arteries known to supply

the infarct. Cerebral infarcts were larger than a single branch artery territory. Cardiac studies showed high or medium risk cardiac sources known to cause embolism. High risk cardiac sources included: valvular surgery; atrial fibrillation (the commonest encountered), atrial flutter or sick-sinus syndrome with or without valvular heart disease; ventricular aneurysm; cardiac mural thrombus; cardiomyopathy; global left ventricular hypokinesis; akinetic ventricular regions; endocarditis; intracardiac tumors. Medium risk sources are: myocardial infarction within 6 months; valvular heart disease without atrial fibrillation; atrial flutter, or sick-sinus syndrome; mitral valve prolapse; mitral annulus calcification; atrial septal defect or patent foramen ovale (paradoxical embolism); hypokinetic ventricular segment.

5. Small Vessel Disease (Perforating Artery Occlusion). Vascular and cardiac studies showed an absence of relevant vascular occlusion/severe stenosis and cardioembolic risks. Cerebral infarct was limited to the territory of one single penetrating branch. Clinically, a neurological deficit must be compatible with a single branch territory infarct.

Non-stroke individuals (NS) were volunteers who had no history of stroke (n=219). The study population was classified as three groups: (1) patients with VAD and PCS (VAD group), (2) patients with PCS of etiologies other than arterial dissection (non-VAD group), and (3) NS. All participants provided informed consent. The Institutional Review Board of TVGH approved the present study.

Vascular Risk Factors Determination

Hypertension was defined as a self-report of current antihypertensive agents use or a measurement of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg on at least two occasions.[22] Diabetes mellitus was defined as a self-report of current glucose-lowering agents use or a measurement of hemoglobin A1c ≥6.5%.[23] Dyslipidemia was defined as a self-report of statin use or a measurement of total cholesterol ≥240 mg/dL.[24]

Measurement of Serum MMP-9 Level

Blood samples were obtained from all participants within 14 days of stroke onset. We drew blood samples in 10 mL EDTA plasma tubes. The blood samples were immediately centrifuged at 363g for 10 minutes. After centrifugation, we stored the samples at −80°C until analysis. ELISA Kits (Cat. No. DY911, DuoSet ELISA, R&D System, Minneapolis, MN, USA) were used to measure MMP-9 levels in the plasma samples, according to the manufacturer's instructions.

Statistical Analysis

Analyses were performed with the SPSS software, version 21 (IBM, Armonk, NY, USA). Variables were expressed as mean±SD, median [interquartile range], or number of patients (%), as appropriate. The χ^2 test was performed for categorical variables. Continuous variables were compared using one-way analysis of variance (ANOVA) with posthoc Tukey test or Student t test, and covariates were included for the comparison of MMP-9. Multivariate logistic regression analyses were used to adjust for the potential confounding factors (including age, sex, hypertension, diabetes mellitus, hyperlipidemia, cigarette smoking, or NIH stroke scale (NIHSS), as specified in the results section) while determining the association between serum MMP-9 level and VAD-PCS. Odds ratios (ORs) with 95% CIs were provided. Statistical significance was set at $P<0.05$ or $P<0.017$ after adjustment for multiple comparisons.

RESULTS

Demographic Comparisons

There were 30 patients with VAD-PCS, 119 patients with PCS of etiologies other than arterial dissection, and 219 NS subjects. Table 1 shows the demographic characteristics of our study population. The etiologies of PCS and patterns of VAD are shown in Table 2. Notably, only one patient with VAD-PCS had extracranial VAD while the others had intracranial VAD.

There were significant differences in age among the three groups (F=11.289, $P<0.001$; Table 1). The age of VAD group was 59.6±15.0 years, which is significantly younger than patients in non-VAD group (69.9±14.0 years, $P<0.001$) but similar to NS group (62.7±14.9 years, $P=0.510$). The proportion of male subjects also differed among the three groups (χ^2=22.662, $P<0.001$; Table 1). Males were significantly more prevalent in the VAD and non-VAD groups than in the NS group ($P<0.001$), but the proportion of male patients did not differ between the two PCS groups ($P=0.103$). As to the prevalence of vascular risk factors, hypertension (χ^2=70.121, $P<0.001$), diabetes mellitus (χ^2=43.701, $P<0.001$), hyperlipidemia (χ^2=54.779, $P<0.001$), and smoking habits (χ^2=27.541, $P<0.001$) were also significantly more common in the VAD and non-VAD groups than in the NS group, but similar between the two PCS groups (hypertension, χ^2=0.637, $P=0.425$; diabetes mellitus, χ^2=0.485, $P=0.486$; hyperlipidemia, χ^2=219, $P=0.640$; smoking habits, χ^2=0.556, $P=0.456$; Table 1).

In patients with PCS, the stroke severity was significantly milder in VAD group compared with non-VAD group (Table 1).

Table 1. Demographics and Characteristics Comparisons of the Study Population

	VAD (n=30)	Non-VAD (n=119)	NS (n=219)	*P* Value
Age, mean±SD, y	59.6±15.0*	69.9±14.0*	62.7±14.9	<0.001
Sex, n (%), male	26 (86.7)†	86 (72.3)‡	114 (52.1)	<0.001
Hypertension, n (%)	20 (66.7)†	88 (73.9)‡	62 (28.3)	<0.001
Diabetes mellitus, n (%)	11 (36.7)†	52 (43.7)‡	27 (12.3)	<0.001
Hyperlipidemia, n (%)	17 (56.7)†	73 (61.3)‡	49 (22.4)	<0.001
Smoking, n (%)	12 (40.0)†	39 (32.8)‡	26 (11.9)	<0.001
MMP9, mean±SD, ng/mL	200.9±81.4*,†	161.3±73.0‡	118.9±55.9	<0.001§
MMP measurement, mean±SD, days from stroke onset	4.3±3.0	4.6±2.8	…	0.60
Initial NIHSS, median [IQR], points	2.0 [3.0]	4.0 [7.0]	…	0.007

IQR indicates interquartile range; MMP, metalloproteinase; NIHSS, National Institutes of Health Stroke Scale; NS, non-stroke; SD, standard deviation; and VAD, vertebral artery dissection.

*P<0.017, VAD vs non-VAD.

†P<0.017, VAD vs NS.

‡P<0.017, non-VAD vs NS.

§Age and sex-adjusted.

Factors Associated With Serum MMP-9 Level

Blood samples were collected within 14 days of stroke onset (mean±SD: 4.5±2.8 days). The time of blood draw between VAD and non-VAD groups were similar (Table 1). The analysis of covariance, with age and sex as covariates, showed that serum MMP-9 levels differed among the three groups (F=29.321, *P*<0.001; Table 1). The results of posthoc analyses showed that both PCS groups had significantly higher serum MMP-9 levels compared with NS group (*P*<0.001); in addition, the serum MMP-9 level in the VAD group was the highest compared with the other two groups (*P*<0.001, versus NS group; *P*=0.01, versus non-VAD group) (Table 1).

Table 2. Stroke Etiologies of Patients With Posterior Circulation Ischemic Stroke (n=149) and Patterns of Vertebral Artery Dissection

Stroke Etiology	Number of Patients (%)
BABO	27 (18.1)
LAA	43 (28.9)
CE	27 (18.1)
SVD	22 (14.8)
VAD	30 (20.1)
One VA involved	26 (86.7)
Bilateral VAs involved	4 (13.3)
Intracranial VA involved	29 (96.7)
BA involvement	6 (20.0)

BA indicates basilar artery; BABO, basilar artery atheromatous branch occlusive disease; CE, cardioembolism; LAA, large artery atherosclerotic stenosis/occlusion; SVD, small vessel disease; VA, vertebral artery; and VAD, vertebral artery dissection.

We further tested the relationship between the serum MMP-9 level and other factors that might be confounding factors contributing to a higher serum MMP-9 level in patients with VAD-PCS. Figure S1 shows that, in the total study population including both patients with PCS and NS individuals, a higher serum MMP-9 level was significantly associated with the presence of PCS (*P*<0.001), male sex (*P*=0.011), and the presence of vascular risk factors, hypertension (*P*<0.001), diabetes mellitus (*P*=0.019), hyperlipidemia (*P*=0.017), and smoking habits (*P*<0.001). In patients with PCS, the results further revealed that the serum MMP-9 level was positively associated with stroke severity (NIHSS; *P*=0.010; Figure S2) and negatively associated with days from stroke onset (*P*=0.006; Figure S2).

VAD was Significantly Associated With Higher Serum MMP-9 Level Independent of Vascular Risk Factors and the Presence of PCS

We then did the multivariate logistic regression analyses to test the independent association between serum MMP-9 levels and VAD. The adjusted variables were factors that were shown to be both significantly associated with serum MMP-9 level (Figures S1 and S2) and different among groups. The results showed that a higher serum MMP-9 level was associated with increased odds of VAD versus control, independent of sex and number of vascular risk factors (OR, 4.572; 95% CI, 2.240–9.333, *P*<0.001; Table 3). In addition, trying to validate the association between VAD and MMP-9 independent of the presence of PCS, we analyzed the odds of VAD versus non-VAD. The results showed that a higher serum MMP-9 level was also

Table 3. Multivariate Logistic Regression Analyses of the Relationship Between Serum MMP-9 Level and Vertebral Artery Dissection With Posterior Circulation Ischemic Stroke (versus Non-Stroke Individuals)

n=247	OR	95% CI	*P* Value
Constant	0.007		<0.001
MMP-9, every 100 ng/mL increment	4.572	2.240–9.333	<0.001
Sex (female)	0.227	0.069–0.744	0.014
Every one vascular risk factor presence	2.185	1.437–3.322	<0.001

Number of vascular risk factors with counts of hypertension, diabetes mellitus, hyperlipidemia and cigarette smoking. MMP indicates metalloproteinase; and OR, odds ratio.

associated with increased odds of VAD, independent of age, sex, and stroke severity (OR, 1.819; 95% CI, 1.034–3.200, *P*=0.038; Table 4).

DISCUSSION

Arterial dissection as the stroke cause is much higher in posterior circulation than in anterior circulation.[25–30] Brainstem infarction caused by VAD could be devastating.[5,6,31] Our previous study of PCS registry also revealed a mortality rate of around 7% in patients with VAD-PCS, which was similar to PCS caused by LAA.[6] Despite its significance in PCS, the mechanism of VAD-PCS is not well understood. Precipitating trauma histories and an underlying connective tissue disease only accounts for <10% of cases.[3,25] The present study could provide insights about its pathophysiology. We found that patients with VAD-PCS had a significantly higher serum MMP-9 level at the acute stage of stroke compared with NS individuals and patients with PCS of other etiologies. Given our previous study showing an elevated leukocyte, particularly neutrophil counts, in patients with VAD-PCS,[7] our results suggest that inflammation-induced MMP-9 escalation might be

Table 4. Multivariate Logistic Regression Analyses of the Relationship Between Serum MMP-9 Level and Vertebral Artery Dissection With Posterior Circulation Ischemic Stroke (versus Patients With Posterior Circulation Ischemic Stroke of Other Etiologies)

n=149	OR	95% CI	*P* Value
Constant	0.815		0.875
MMP-9, every 100 ng/mL increment	1.819	1.034–3.200	0.038
Age, y	0.963	0.934–0.994	0.019
Sex (female)	2.069	0.633–6.763	0.229
NIHSS (point)	0.932	0.862–1.008	0.077

MMP indicates metalloproteinase; NIHSS, National Institutes of Health Stroke Scale; and OR, odds ratio.

involved in the pathophysiology of VAD with ischemic events.

MMP-9 has been found to be upregulated in response to ischemic stroke itself.[17–19] Several studies have found that serum MMP-9 level is associated with stroke severity and infarct volume at the acute stage of ischemic stroke.[17,18] However, few have regarded the effect of stroke etiology on these associations. Our results, echoing the previous studies, demonstrated a higher serum MMP-9 level in patients with PCS compared with NS individuals. We further showed that patients with PCS caused by VAD had significantly higher serum MMP-9 levels compared with PCS of other etiologies, which was independent of age, sex, and stroke severity. These results support the idea that an elevated serum MMP-9 level in VAD-PCS is not solely caused by ischemic stroke itself or other confounding factors but might also be associated with the pathophysiology of vascular damage in VAD.

MMP-9 is an inducible ECM-degrading enzyme and is usually involved in inflammatory process, where its expression is induced by cytokines, such as IL-1β, TNF-α, and IL-6, and reduced by anti-inflammatory molecules, such as IL- 4, IL-10, interferon β, retinoids, and glucocorticosteroids.[11–13] Thus, not surprisingly, MMP-9 has been found mediating several arterial diseases involving inflammation processes, such as atherosclerotic plaque rupture,[10] arterial stiffness,[32] giant cell arteritis,[33] aortic aneurysm,[34–36] and acute thoracic aorta dissection.[16] In studies of aortic aneurysm, accumulating data generally support the paradigm that the initial tissue injury during acute aortic dissection triggers a cycle of inflammation, MMP-9 overproduction, and progressive destruction of the aortic wall, ultimately leading to expansion and rupture.[14,34–36] Our previous study shows that inflammation and neutrophil-mediated process might be involved in the mechanism of VAD-PCS.[7] MMP-9 is primarily secreted from immune cells, including neutrophils, under inflammation processes.[11–13] In an animal model of aortic dissection, MMP-9 secreted from neutrophils infiltrating the aortic intima triggers the acute aortic dissection.[16] We suggest that MMP-9 secreted from leukocytes and MMP-9-mediated vascular injury might also be involved in the pathophysiology of VAD with PCS. However, more basic studies are needed to validate the definite molecular mechanisms involved in VAD-PCS.

There are anatomic factors and locations susceptible to cervicocephalic arterial dissection. In most study series, intracranial artery dissections affect the posterior circulation more frequently than the anterior circulation.[25–30] A case-control study shows that hypoplastic VAs are more prone than dominant VAs to dissection.[37] Since circulatory leukocytes, particularly neutrophils, adhere endothelial cells more easily in areas with lower shear stress,[38] our postulated mechanism may explain

why cervicocephalic arterial dissections are more prone to occur in the posterior circulation and hypoplastic arteries, which have a lower flow.

There are previous studies evaluating the relationship between cervical artery dissection and MMP-9. One study tested two MMP-9 DNA polymorphisms and showed no difference in the allelic distribution of either polymorphism between patients with cervical artery dissection and normal controls.[39] The other study measured serum MMP-9 level at the chronic stage (3 months after) of vascular event and also found no difference between patients with cervical artery dissection and ischemic stroke of other causes.[40] In these studies, most cases are carotid arterial dissection rather than VAD.[39,40] Meanwhile, almost all of our VAD patients were intracranial VAD (29/30). Apart from different vascular locations of dissection in these studies, inconsistent results might indicate that MMP-9 is only involved at the acute stage of arterial dissection with ischemic stroke and further support our postulation: abnormal MMP-9 elevation being inflammation-induced but not attributable to hereditary or constitutional conditions in the mechanism of VAD-PCS.

The therapeutic potential of MMP-9 inhibition has been shown mostly in cerebral infarctions. MMP-9 inhibition treatment at the early stage of stroke could reduce infarct volume[17,41] and prolong the thrombolytic time window in experimental stroke.[42] Specific or non-specific MMP-9 inhibition has also been shown to decrease incidence and severity of aortic dissection in murine models.[16,43] Since the present study has provided evidence of MMP-9 associating with VAD-PCS, it is reasonable to test whether MMP-9 inhibition could also decrease the severity of VAD and/or related PCS severity at the acute stage in the future.

In addition to MMP-9, there are other study targets for VAD-PCS biomarkers. These candidates include those associated with aortic dissection, such as vascular endothelial growth factor, fibrillin-1,[44] E-prostanoid receptor 4 (receptor of prostaglandin E2),[45] and NLRP3–caspase-1 inflammasome.[46] Among them, serum level of Fibrillin-1, in particular, has also been shown to be higher in patients with spontaneous cerebral artery dissection during the acute stage of stroke.[47] Fibrillin-1 is a calcium-binding protein that assembles to form 10 to 12 nm microfibrils in the ECM.[48] Whether and how MMP-9 and fibrillin-1 are involved together or interact in the pathophysiology of VAD-PCS warrant further investigations.

There are limitations in the present study. Firstly, our recruited VAD patients numbers were few and all presented with PCS. Our results might only apply to VAD with ischemic stroke but not with its other clinical presentations, such as subarachnoid hemorrhage or isolated neck pain/headache. Secondly, the cross-sectional clinical study settings revealing the associations between VAD and serum MMP-9 level could not validate their causal relationship yet. In addition, we obtained the serum MMP-9 level after VAD or non-VAD stroke events, as opposed to obtaining the baseline level before events. Though we have adjusted the PCS itself in the association analyses, we still could not exclude serum MMP-9 level increments as a response rather than etiology of VAD-PCS. We would need an animal model to test our postulated pathophysiology or even explore the potential effective treatments, such as MMP-9 inhibitors or anti-inflammatory agents, for VAD-PCS. Thirdly, we did not analyze the relationship between serum MMP-9 level and functional outcomes in our patients because of the small sample size, where the type II error would occur. Further study with a larger size of VAD population with varied clinical presentations will be needed. Fourthly, it has been shown that statins, simvastatin in particular, and non-steroidal anti-inflammatory drugs (NSAIDs) can decrease the synthesis or activity of MMP-9.[49–51] Almost all patients in our stroke registry would be prescribed statins at and during hospitalization; however, we were unable to provide the information of statin kinds and NSAIDs prescription. Since simvastatin decreases serum MMP-9 levels at more than one month (45 days) of treatment, it should not affect our analyzed results at the earlier period of stroke.[49,50] Lastly, we did not quantitate the infarct volume, which might be positively associated with serum MMP-9 level in PCS.[52] In our study, VAD group had a lower NIHSS and was presumably a smaller infarct volume than non-VAD group; a higher serum level of MMP-9 might be underestimated in VAD group. However, since NIHSS is not as valid in PCS to reflect infarct severity as in anterior circulation ischemic stroke,[53] the relationship between infarct volume and serum MMP-9 level in PCS needs further study to validate.

CONCLUSIONS

Patients with VAD had higher levels of serum MMP-9 at the acute stage of PCS compared with NS individuals and PCS of other causes. Our results support the hypothesis of the involvement of ECM-degrading protease in the mechanism of VAD, particularly intracranial VAD.

Affiliations

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Disclosures

None.

## Supplementary Materials

Figures S1–S2

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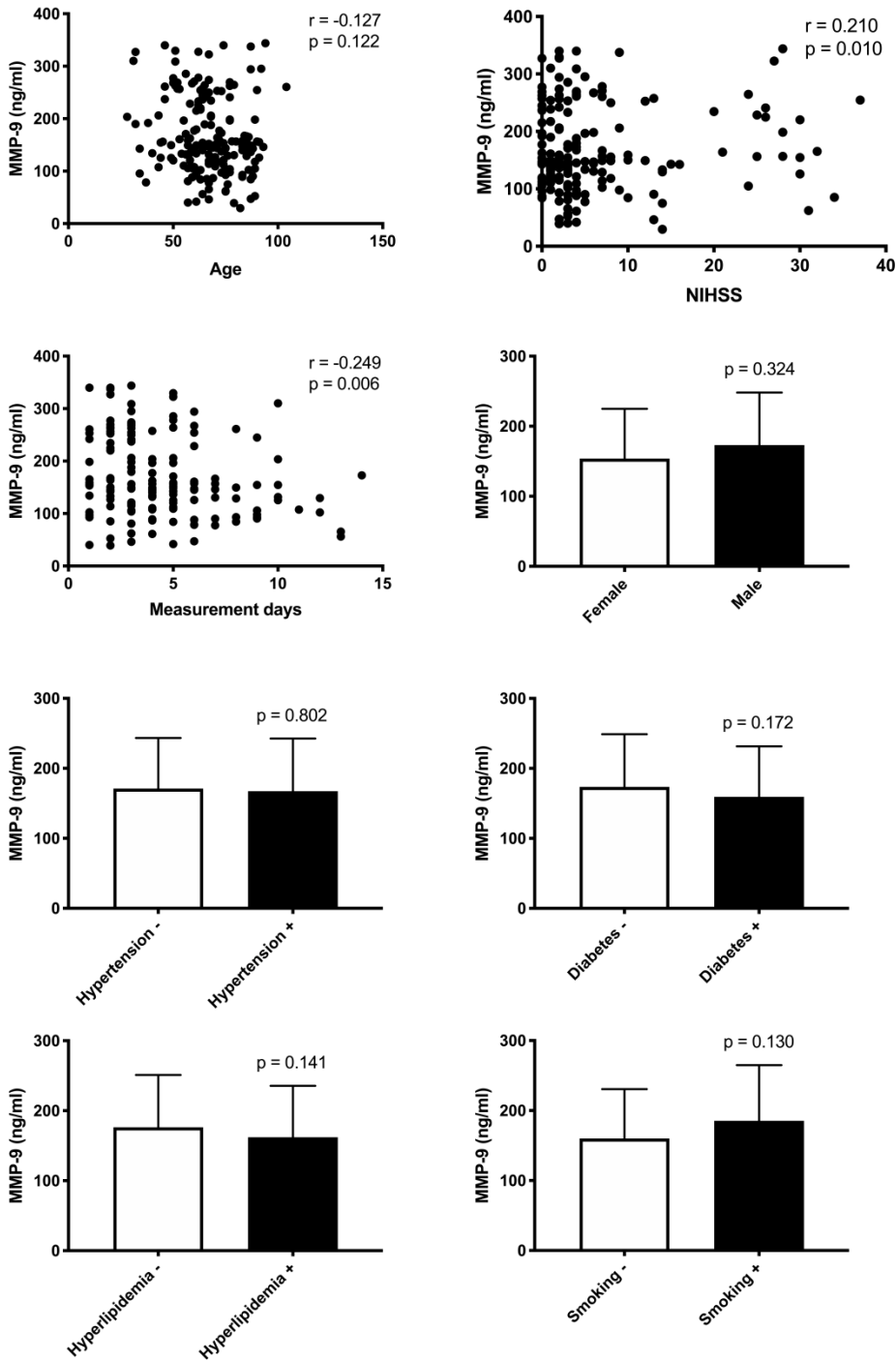
SUPPLEMENTAL MATERIAL

Figure S1. Factors associated with serum MMP-9 levels in the total study population.
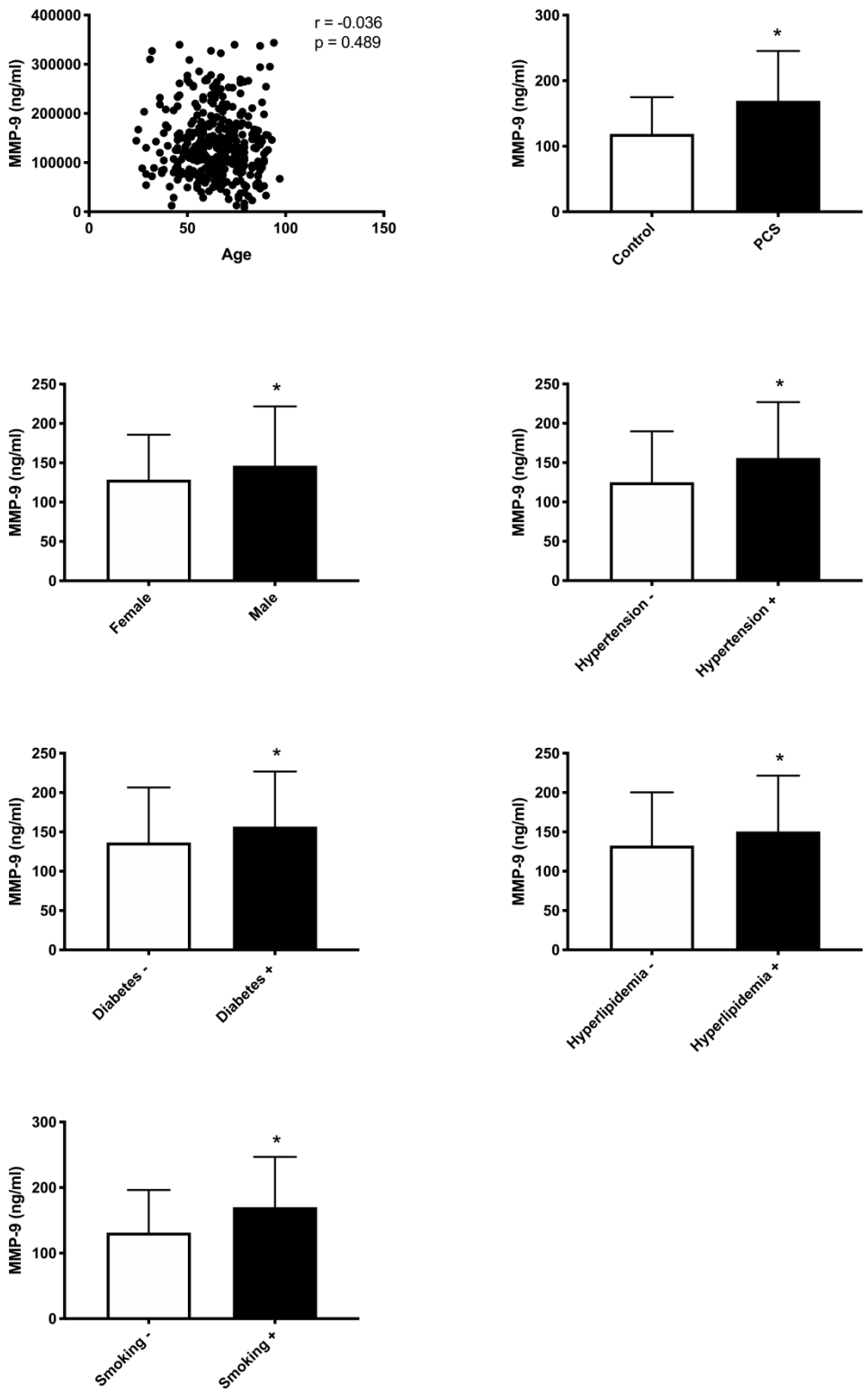
*p < 0.05

Figure S2. Factors associated with serum MMP-9 levels in patients with posterior circulation ischemic stroke.

*p < 0.05