High Serum Level of Matrix Metalloproteinase-7 Is Associated With Increased Risk of Spontaneous Subarachnoid Hemorrhage

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Background and Purpose—Increased degradation of the extracellular matrix in the arterial wall by matrix metalloproteinases (MMPs) may be an important mechanism in the pathogenesis of intracranial aneurysms and subarachnoid hemorrhage (SAH). MMP-2 and MMP-9 have been suggested to be involved in matrix degradation preceding SAH. We studied serum levels of MMP-1, -2, -3, -7, -9, -10, and -12 and the risk of incident SAH.

Methods—A nested case–control study within the population-based cohort, Malmö Diet and Cancer study, was performed including incident cases of spontaneous SAH (n=79) and controls matched by age, sex, and follow-up time (n=232). MMPs were measured in serum from the baseline examination in 1991 to 1996. MMPs were compared between cases and controls, using conditional logistic regression adjusting for risk factors.

Results—Baseline levels of MMP-7, MMP-10, and MMP-12 were significantly higher in incident SAH cases compared with controls. Odds ratios (95% confidence interval) for SAH per 1 SD increase of MMP-7, MMP-10, and MMP-12 were 1.78 (1.31–2.41), 1.45 (1.11–1.91), and 1.53 (1.17–2.01), respectively. After adjustment for SAH risk factors, MMP-7 was still significantly associated with SAH (odds ratio: 1.64; 95% confidence interval: 1.19–2.27; P=0.0026), whereas associations for MMP-10 and MMP-12 were attenuated and nonsignificant. We did not find any association between high serum levels of MMP-2 or MMP-9 and SAH risk.

Conclusions—High serum level of MMP-7 was associated with increased risk of incident spontaneous SAH, independently of the main risk factors for SAH. High serum levels of MMP-2 and MMP-9 did not predict SAH risk. **(*Stroke*. 2018;49:1626-1631. DOI: 10.1161/STROKEAHA.118.020660.)**

Key Words: case-control studies ■ matrix metalloproteinases ■ odds ratio ■ risk factors ■ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) is a devastating disease associated with a high mortality and a high risk of permanent severe functional deficits in survivors. Disruption of the extracellular matrix (ECM) in the arterial wall is thought to be an important mechanism in the formation and rupture of intracranial aneurysms, which is the main underlying cause of SAH.[1,2] The ECM provides the integrity and strength of the arterial wall, and exaggerated breakdown of its main components, collagen, elastin, proteoglycans, and glycoproteins, may reduce elasticity and strength and thus increase susceptibility to focal arterial dilation, aneurysm formation, and rupture.[3]

Matrix metalloproteinases (MMPs) are essential in tissue remodeling in developing and regenerative processes. Several MMPs can degrade important ECM components in the arterial wall. For example, MMP-2, -7, -9, and -12 can degrade insoluble elastin, which is responsible for most of the elasticity in the arterial wall.[4] MMPs also contribute to ECM disruption and remodeling in more complex pathways.[3] Increased ECM remodeling is observed in both unruptured and ruptured aneurysms,[2] and studies of intracranial aneurysms have specifically suggested important contribution of MMP-2 and MMP-9 to the formation and rupture of aneurysms.[5–9]

It is unclear whether systemic levels of MMPs are associated with the future risk of spontaneous SAH. Circulating MMPs may serve as systemic markers of vascular ECM remodeling and, hypothetically, could be used to predict the risk of SAH in individuals with intracranial aneurysms or high risk for SAH. The discovery of new biomarkers may also provide hypotheses for studies evaluating potential preventative treatments. In this study, we evaluated serum levels of MMP-2 and -9, and also MMP-1, -3, -7, -10, and -12, in relation to the risk of future spontaneous SAH in a population-based study.

Methods

The data that support the findings of this study are available after application to the MDC (Malmö Diet and Cancer) study steering committee at Lund University, Sweden.

Received January 4, 2018; final revision received April 19 2018; accepted May 4, 2018.

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The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.118.020660/-/DC1.

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Study Population

The MDC study is a population-based cohort study performed in the city of Malmö, Sweden. Between 1991 and 1996, all men aged 46 to 73 years and all women aged 45 to 73 years, living in Malmö, were invited to the baseline examinations.[10] A total of 28 449 individuals (11 246 men and 17 203 women) were included, out of an eligible population of ≈69 000. All participants gave informed consent. The study was approved by the ethics committee at Lund University (LU51/90, 166/2007, 633/2009, 566/2013).

Baseline Examinations

At the baseline examination, all participants underwent a physical examination and filled in a self-administered questionnaire, and venous blood samples were drawn. Blood pressure was measured in the supine position after 10 minutes of rest. Body mass index and waist circumference were measured using standard methods. Information about smoking habits, alcohol intake, and current medication was assessed in a questionnaire. For the present study, smoking status was categorized into current smoker (regular or occasional smoker) or nonsmoker (former or never smoker). High alcohol intake was defined as >40 g/d for men and >30 g/d for women. White blood cell count (WBC) was determined in fresh blood using automated counters in the hospital laboratory.

Subarachnoid Hemorrhage

All participants in the MDC were followed-up from the baseline examination until a spontaneous SAH event, death, emigration from Sweden, or December 31, 2010, whichever occurred first. Spontaneous, nontraumatic SAH cases were identified in the Malmö Stroke Register, and the Swedish Inpatient and Causes of Death Registers (*International Classification of Diseases Ninth and Tenth Revision* codes 430 and I60), by methods that have been described previously.[10,11] Diagnosis codes for traumatic SAH were not included. Also, cases were excluded if trauma were thought to have caused the hemorrhage based on the patient history and imaging at validation of medical records. Seventy-one of the 79 incident SAH cases in this study were either confirmed by review of hospital records, confirmed by autopsy, or discharged with a diagnosis of SAH from a neurosurgical clinic. We did not have information about whether the SAH was aneurysmal or nonaneurysmal for the whole sample.

Nested Case–Control Study

A nested case–control study within the MDC cohort was constructed using the incidence-density sampling method, after excluding subjects with preexisting SAH at baseline. Cases were individuals diagnosed with SAH during follow-up (incident SAH). The date of SAH diagnosis was considered the index date. For each case, 3 to 4 controls, still alive and free from SAH on the index date, were randomly selected from the MDC, matched on sex and age (5-year groups). Eighty-six incident cases and 276 controls were initially identified for this study; after exclusion of samples with technical laboratory errors or too small volumes of serum, 79 cases and 261 controls remained. Because of the matched design, an additional 29 controls were removed from the analysis because the corresponding cases were excluded. The study sample thus consisted of 79 cases (mean age, 58 years; 72% women) and 232 controls (mean age, 58 years; 74% women). Median time from baseline to the SAH event was 8.5 years (range, 0.4–18.7 years).

Laboratory Analysis of MMPs

Nonfasting blood samples were drawn at the baseline examinations between 1991 and 1996, and serum was separated within 1 hour. The samples were then frozen at −80°C until MMP analyses.[12] Levels of MMP-1, -3, -7, -10, and -12 were analyzed in serum from SAH cases and control subjects, using the Olink CVD panel (Olink Bioscience, Uppsala, Sweden) based on the Proximity Extension Assay technology.[13,14] Analyses were conducted at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala, Sweden. The intra-assay coefficients of variation for MMP-1, -3, -7, -10, and -12 were 5%, 7%, 11%, 7%, and 9%, respectively. The interassay coefficients of variation were 31%, 20%, 16%, 15%, and 39%, respectively. Protein concentrations were expressed in normalized protein expression units (real-time polymerase chain reaction quantification cycle values on the log2-scale).[14] Detailed information about the assay is provided at the Olink website.[14]

MMP-2 and -9 were analyzed with an ELISA assay (R&D systems, https://www.rndsystems.com/products/human-magnetic-luminex-assay_lxsahm). Concentrations of MMP-2 and MMP-9 are expressed in pg/mL. In MMP-2 analyses, 72 cases and 226 controls were included, and in MMP-9 analyses, 79 cases and 226 controls.

Statistical Analysis

Distributions of MMPs were right-skewed, and concentrations were natural log-transformed. Correlations between MMPs were determined by Pearson correlation coefficients. The associations between MMPs and baseline risk factors were evaluated using linear regression.

Conditional logistic regression models, taking into account each set of matched cases and controls, were used to evaluate the associations between each MMP (per SD increment) and SAH. Because incidence-density sampling was used for selection of controls, this analysis will estimate the incidence rate ratio. Models were adjusted for SAH risk factors (current smoking, systolic blood pressure, blood pressure treatment, body mass index, diabetes mellitus, and high alcohol intake). In a second adjusted model, we added WBC to the covariates because WBC was associated with some of the MMPs (Table I in the online-only Data Supplement) and has also been associated with SAH risk.[11] We also explored whether former smoking influenced the results, by including categories of never, former, and current smokers in the model. This did not substantially change the associations between MMPs and SAH, and the variable with current and nonsmokers was, therefore, used. A multiplicative interaction term was added to the adjusted model to evaluate any effect modification of smoking on the associations between MMP-7, MMP-10, and MMP-12 and SAH. Seven proteins were examined, and multiple testing was accounted for by Bonferroni correction, setting the significance level to $P<0.007$ (0.05/7).

Results

Baseline Characteristics and MMP Levels

Baseline risk factors and MMP levels in cases and controls are shown in Table 1. Incident SAH cases had higher prevalence of smoking, and higher blood pressure and WBC, compared with controls. Associations between MMPs, and between MMPs and risk factors, are shown in Tables I and II in the online-only Data Supplement. There were significant correlations for all pairwise combinations of MMP-3, -7, -9, -10, and -12 with r values of 0.17 to 0.58 (all $P<0.003$) except for MMP-3 and -9 ($r=0.10$; $P=0.074$; Table I in the online-only Data Supplement). MMP-3, -7, and -12 were positively associated with systolic and diastolic blood pressures. Higher levels of MMP-9, -10, and -12 were associated with smoking and higher WBC. MMP-7 was also positively associated with WBC, and with body mass index, but not significantly with smoking (Table 2; Table II in the online-only Data Supplement).

Associations Between MMPs and SAH

Levels of MMP-7, MMP-10, and MMP-12 were significantly higher in spontaneous SAH cases compared with controls in unadjusted models ($P\leq0.007$; Tables 1 and 3). For MMP-7,

Table 1. Cardiovascular Risk Factors and Levels of Matrix Metalloproteinases in SAH Cases and Controls

	Cases	Controls
n	79	232
Age, y, mean (SD)	58 (8)	58 (8)
Women, n (%)	57 (72)	171 (74)
Born in Sweden, n (%)	69 (87)	204 (88)
Born outside Europe, n (%)	0 (0)	1 (0.4)
Systolic BP, mm Hg, mean (SD)	143 (17.6)	137 (19.6)
Diastolic BP, mm Hg, mean (SD)	87 (9.0)	84 (9.5)
BP drug, n (%)	16 (20)	28 (12)
Body mass index, kg/m^2, mean (SD)	24.7 (4.3)	25.0 (3.7)
Current smoking, n (%)	40 (51)	65 (28)
Diabetes mellitus, n (%)	3 (4)	4 (2)
High alcohol intake, %	1 (1)	10 (4)
White cell blood count, 10^9/L, mean (SD)	6.8 (1.8)	6.1 (1.7)
Prior cardiac event or stroke, n (%)	6 (7.6)	2 (0.9)
Prior stroke (not SAH), n (%)	4 (5)	1 (0.4)
MMP-1, median (IQR)	14.5 (18.0)	14.8 (19.5)
MMP-2, median (IQR)	556 (242)	595 (259)
MMP-3, median (IQR)	2.42 (1.24)	2.25 (1.21)
MMP-7, median (IQR)	1710 (791)	1404 (645)
MMP-9, median (IQR)	201 (133)	181 (129)
MMP-10, median (IQR)	155 (11)	131 (90)
MMP-12, median (IQR)	183 (148)	159 (99)

BP indicates blood pressure; IQR, interquartile range; MMP, matrix metalloproteinases; and SAH, subarachnoid hemorrhage.

there was strong evidence of a positive association between baseline serum concentrations and SAH incidence (odds ratio [OR] per SD increase on the log scale: 1.78; 95% confidence interval: 1.31–2.41; P=0.00021), and this association remained statistically significant even after adjusting for risk factors (OR: 1.64; 95% confidence interval: 1.19–2.27; P=0.0026). The associations of MMP-10 and MMP-12 with SAH were weakened and nonsignificant after adjusting for risk factors (P>0.01; Table 3). The reduced OR was mainly explained by adding smoking to the covariates. There was no effect modification by smoking for the associations between MMP-7, -10, or -12 with SAH (P>0.5), that is, we found no evidence of different associations in smokers and nonsmokers.

MMP-9 levels were slightly, but not significantly, higher in cases than controls, and this difference was even smaller after adjusting for smoking (OR: 1.04; 95% confidence interval: 0.78–1.41; Tables 1 and 3). MMP-2 tended to be lower in cases than controls, and there was a suggested negative association after adjustment for risk factors (OR: 0.73; 95% confidence interval: 0.54–0.98).

Discussion

In this study, MMP levels measured in serum were evaluated in relation to risk of future spontaneous SAH in subjects from the general population. Degradation of ECM has been suggested as an important mechanism in the pathogenesis of intracranial aneurysms and SAH. Previous studies have observed increased activity of MMP-2 and MMP-9 in intracranial aneurysms,[5,15,16] and we hypothesized that MMP-2 and MMP-9, or other MMPs with influence on ECM degradation, could be elevated also in the systemic circulation of subjects who later developed SAH. High systemic levels of MMP-2 or MMP-9 were, however, not associated with increased risk of SAH in the present study. Instead, we found that high levels of MMP-7 were strongly associated with risk of SAH, also when adjusting for risk factors.

Several observations support a role of impaired ECM structure in the pathogenesis of intracranial aneurysms and SAH. SAH risk is increased in several heritable disorders affecting the connective tissue, in particular collagen, for example, polycystic kidney disease and Ehler–Danlos syndrome type IV. However, these conditions are rare and cause only a minority of SAHs.[17] Although the synthesis of ECM components, for example, collagen III, does not seem to be impaired in patients with aneurysms in general,[18] studies have observed signs of increased enzymatic degradation of ECM components, both in the circulation from patients with aneurysms and in aneurysm tissue.[6,18,19] Plasma elastase levels were elevated in patients with ruptured and unruptured aneurysms compared with controls in 2 studies.[6,19] Other studies have shown that the expression and activity of MMP-2 and -9 are increased in human intracranial aneurysm tissue, compared with normal intracranial arteries, and also that the MMP inhibitor activity is higher.[5,9,15,16] In a study of resected human aneurysms, it was reported that gene expression of MMP-2 and -9 was increased in ruptured compared with unruptured aneurysms.[20] Research using animal models of aneurysms and SAH has also indicated important roles of ECM degradation by MMPs, in particular MMP-2 and MMP-9.[7,8] For example, in a mouse model, Aoki et al[8] showed that MMP-2 and MMP-9 were involved in aneurysm progression, which could be partly reduced by an MMP-2, -9, and -12 inhibitor.

In the present study, we did not find any clear association between high serum levels of MMP-2 or MMP-9 and SAH risk. In one previous study, activity of MMP-9 in serum was not higher in 6 patients with aneurysms compared with 6 control subjects.[9] This is in line with our results. Based on these and our results, it seems possible that local MMP-2 and -9 activity in aneurysms is not reflected by the systemic levels, and these MMPs might be less useful as prospective systemic biomarkers of SAH or aneurysms. Another study found increased serum levels of MMP-2 and -9 in patients with ruptured compared with unruptured aneurysms, in serum samples taken at aneurysm surgery.[20] It was not evaluated whether levels were increased before the SAH. Further studies in larger samples of patients with unruptured intracranial aneurysms are needed to study if serum levels of MMP-2 and -9 still may predict rupture and SAH, perhaps during a shorter period of time before the SAH.

There are several plausible mechanisms through which MMP-7 could be involved in the pathogenesis of SAH. MMP-7 (matrilysin) is together with MMP-12 (macrophage elastase) and gelatinases, that is, MMP-2 and -9, capable

Table 2. Association Between Quartiles of MMP-7, Baseline Risk Factors, and Risk of SAH

	Quartile-1	Quartile-2	Quartile-3	Quartile-4	
n	77	77	78	79	
n cases/controls	13/64	14/63	21/57	31/48	
MMP-7, range	760–1184	1193–1479	1489–1885	1898–3259	
MMP-7, median (IQR)	1053 (193)	1305 (100)	1658 (185)	2150 (382)	
Women, n (%)	52 (68)	58 (75)	58 (74)	60 (76)	
Current smoker, n (%)	24 (31)	23 (30)	26 (33)	32 (41)	
Former smoker, n (%)	24 (31)	24 (31)	23 (29)	19 (24)	
Never smoker, n (%)	29 (38)	30 (39)	29 (37)	28 (35)	
Diabetes mellitus, n (%)	2 (3)	0 (0)	3 (4)	2 (3)	
High alcohol intake, n (%)	5 (6)	3 (4)	1 (1)	2 (3)	
Age, mean (SD)	56 (6.8)	56 (7.5)	58 (8.3)	61 (7.8)	
Systolic BP, mm Hg, mean (SD)	134 (16)	138 (20)	140 (21)	143 (18)	
Diastolic BP, mm Hg, mean (SD)	83 (8.8)	85 (9.9)	85 (10.4)	86 (8.7)	
BMI, kg/m^2, mean (SD)	24.0 (3.1)	25.0 (4.1)	25.1 (4.1)	25.6 (3.9)	
WBC, mean (SD)	5.8 (1.4)	6.2 (2.0)	6.3 (1.6)	6.9 (1.8)	
					P trend
OR for SAH (95% CI)	1	0.93 (0.40–2.20)	1.99 (0.89–4.43)	3.71 (1.67–8.24)	0.00032
OR for SAH (95% CI)*	1	0.82 (0.33–2.02)	1.84 (0.78–4.33)	3.03 (1.28–7.15)	0.0040

BMI indicates body mass index; BP, blood pressure; CI, confidence interval; IQR, interquartile range; MMP, matrix metalloproteinases; OR, odds ratio; SAH, subarachnoid hemorrhage; and WBC, white blood cell count.

*Adjusted for smoking, systolic blood pressure, blood pressure drug, diabetes mellitus, body mass index, and high alcohol intake.

of degrading insoluble elastin.[4] In studies of atherosclerotic plaques, MMP-7 secreted by macrophages can cleave elastin, proteoglycans, versican, and fibronectin in the fibrous cap causing plaque destabilization and rupture.[21,22] In patients with carotid plaques, levels of MMP-7 both in plasma and in the plaque were highest in those with the most recent clinical symptoms, thus reflecting the plaques with highest risk of rupture.[22] Serum MMP-7 levels are also increased in patients with abdominal aortic aneurysms.[23] In patients with diabetes mellitus, MMP-7 measured in plasma was associated with higher degree of atherosclerosis and higher incidence of coronary events.[24] MMP-7 also induces vascular smooth muscle cell apoptosis, which is thought to promote plaque instability and rupture in atherosclerosis.[25] In intracranial aneurysm pathogenesis, apoptosis of smooth muscle cells is an important characteristic especially in aneurysms that have thin walls and are prone to rupture.[2] Loss of mural smooth muscle cells may be a key marker for the change from a stable aneurysm to progression toward rupture.[2] Increased activity of MMP-7 could hence contribute to increased SAH risk.

Studies of animal models, or studies using so called Mendelian randomization, could be performed to further

Table 3. Associations Between MMPs and Incidence of SAH

	OR (95% CI)	*P* Value	Adjusted* OR (95% CI)	*P* Value	Adjusted† OR (95% CI)	*P* Value
MMP-1	0.89 (0.68–1.15)	0.361	0.90 (0.68–1.18)	0.439	0.87 (0.65–1.15)	0.321
MMP-2	0.77 (0.59–1.01)	0.058	0.73 (0.54–0.98)	0.034	0.75 (0.56–1.02)	0.064
MMP-3	1.25 (0.94–1.66)	0.132	1.26 (0.91–1.72)	0.157	1.20 (0.86–1.68)	0.275
MMP-7	1.78 (1.31–2.41)	0.00021	1.64 (1.19–2.27)	0.0026	1.60 (1.16–2.23)	0.0046
MMP-9	1.23 (0.93–1.62)	0.139	1.06 (0.79–1.41)	0.713	0.88 (0.63–1.24)	0.474
MMP-10	1.45 (1.11–1.91)	0.0072	1.39 (1.04–1.87)	0.028	1.33 (0.99–1.80)	0.062
MMP-12	1.53 (1.17–2.01)	0.002	1.43 (1.07–1.92)	0.017	1.35 (0.99–1.85)	0.056

CI indicates confidence interval; MMP, matrix metalloproteinases; OR, odds ratio, per SD increase of MMP on the log scale; and SAH, subarachnoid hemorrhage.

*Adjusted for smoking, systolic blood pressure, blood pressure drug, diabetes mellitus, body mass index, and high alcohol intake.

†Additionally adjusted for white cell blood count.

support a role of MMP-7 in the pathogenesis of intracranial aneurysms and SAH. Also, studying MMP-7 in relation to other aneurysmal vascular diseases, such as aortic aneurysms, could gain more insight into the pathophysiology. For the potential clinical utility, further studies investigating whether MMP-7 levels can predict SAH risk in patients with known aneurysms would be of interest. For example, it could be studied if adding MMP-7 levels to other factors to predict risk of aneurysm rupture could improve clinical prediction of rupture risk, and if this could be used when deciding on whether preventive intervention of an aneurysm should be recommended.

MMP-7 has also been strongly related to pulmonary fibrosis, and serum MMP-7 has been suggested as a potential clinical biomarker for idiopathic pulmonary fibrosis.[26] The risk of SAH has been shown to be higher in subjects with reduced lung function independently of smoking.[27] It could be speculated that increased MMP-7 activity may influence pathological processes in both lung tissue and arterial walls, and thus could be one potential explanation for the association between lung function and SAH.

The roles of MMP-10 and MMP-12 in SAH pathogenesis are unclear. Because the majority of inflammatory cells detected in aneurysm tissue are macrophages, it has been hypothesized that MMP-12, which is secreted by macrophages, may have an important role in SAH pathogenesis.[28] Serum levels of MMP-10 and -12 were significantly higher in incident SAH cases compared with controls in the present study. However, these associations were attenuated and nonsignificant after adjusting for risk factors. MMP-10 and -12 may still mediate some of the effects of smoking on SAH risk, and thus be risk factors for SAH in smokers. However, a larger study is needed to address this question by performing subgroup analyses with higher number of cases in each subgroup.

Strengths and Limitations

The study was a nested case–control study within the well defined MDC cohort, and the risk of selection bias is low because cases and controls come from the same cohort. The MDC cohort is large and the follow-up time relatively long, and therefore the number of SAH cases is reasonable. There are few population-based cohort studies with biomarker levels measured before the SAH event.

The number of SAH cases is still low, and this limits the possibility of performing subgroup analyses. Results need to be confirmed in other studies and in a larger sample of confirmed aneurysmal SAH. The present study population is predominantly of white (Northern European) ancestry. Studies of subjects with other ethnicities would also be of interest because levels of some biomarkers and their influence on disease may differ according to ethnicity. However, we do not have any specific reason to think that this would be the case for MMP-7 and SAH.

In conclusion, baseline serum levels of MMP-7 were clearly higher in subjects with incident spontaneous SAH compared with controls. It should be further investigated whether MMP-7 is involved in the pathogenesis of SAH or could be used as a predictive marker for SAH, for example, in patients with prevalent unruptured intracranial aneurysms.

Acknowledgments

The Science for Life laboratory at Uppsala, Sweden, is acknowledged for protein analyses.

Sources of Funding

Funds from the Swedish Stroke foundation, Sparbanksstiftelsen Färs & Frosta, and the Swedish Heart and Lung foundation supported this study.

Disclosures

None.

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High Serum Level of Matrix Metalloproteinase-7 Is Associated With Increased Risk of Spontaneous Subarachnoid Hemorrhage

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Stroke. 2018;49:1626-1631; originally published online June 7, 2018;
doi: 10.1161/STROKEAHA.118.020660
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0039-2499. Online ISSN: 1524-4628

SUPPLEMENTAL MATERIAL

High serum level of matrix metalloproteinase-7 is associated with increased risk of spontaneous subarachnoid hemorrhage.

Supplemental Table I. Pairwise correlations (Pearsons correlation coefficient, r) between MMPs (natural log transformed) and continuous risk factors.

		MMP-1	MMP-2	MMP-3	MMP-7	MMP-9	MMP-10	MMP-12
MMP-1								
MMP-2	r	0.13						
	p	0.02						
MMP-3	r	-0.06	-0.05					
	p	0.31	0.34					
MMP-7	r	-0.01	-0.049	0.33				
	p	0.79	0.38	<0.0001				
MMP-9	r	0.12	0.07	0.19	0.10			
	p	0.036	0.19	0.0008	0.074			
MMP-10	r	-0.018	-0.14	0.27	0.41	0.20		
	p	0.74	0.009	<0.0001	<0.0001	0.0003		
MMP-12	r	-0.027	0.04	0.23	0.54	0.17	0.30	
	p	0.62	0.46	<0.0001	<0.0001	0.0026	<0.0001	
WBC	r	0.03	-0.17	0.16	0.20	0.58	0.24	0.28
	p	0.53	0.0017	0.0032	0.0003	<0.0001	<0.0001	<0.0001
Age	r	-0.11	0.012	0.058	0.23	-0.17	-0.09	0.26
	p	0.04	0.82	0.29	<0.0001	0.0018	0.11	<0.0001
Body mass index	r	-0.033	-0.12	0.06	0.16	0.04	-0.07	0.12
	p	0.54	0.027	0.24	0.0033	0.46	0.21	0.023
Systolic BP	r	-0.05	-0.029	0.15	0.18	-0.046	-0.04	0.13
	p	0.36	0.59	0.006	0.002	0.39	0.41	0.018
Diastolic BP	r	0.002	0.019	0.19	0.12	-0.005	0.02	0.076
	p	0.97	0.73	0.0004	0.027	0.93	0.71	0.16

WBC, white blood cell count; BP, blood pressure.

Supplemental Table II. Simple linear regression of protease levels (dependent variable) and risk factors.

	MMP-1, beta (95%CI)	p	MMP-2, beta (95% CI)	P	MMP3, beta (95%CI)	P	MMP7, beta (95%CI)	p
Sex	0.13 (-0.11-0.37)	0.286	0.21 (-0.03-0.44)	0.089	-1.15 (-1.36- -0.94)	<0.001	0.13 (-0.11-0.37)	0.286
Diabetes	0.23 (-0.48-0.93)	0.522	-0.25 (-0.89-0.38)	0.429	0.64 (-0.06-1.34)	0.074	0.23 (-0.48-0.93)	0.522
Smoking	0.10 (-0.12-0.33)	0.368	-0.08 (-0.30-0.15)	0.506	-0.13 (-0.36-0.09)	0.244	0.10 (-0.12-0.33)	0.368
High alcohol consumption	-0.56 (-1.13-0.02)	0.058	-0.03 (-0.57-0.50)	0.904	-0.02 (-0.60-0.56)	0.956	-0.56 (-1.1-0.02)	0.058
WBC	0.02 (-0.04-0.08)	0.531	-0.01 (-0.16- -0.04)	0.002	0.09 (0.03-0.15)	0.003	0.11 (0.05-0.17)	<0.001
BMI	-0.009 (-0.04-0.02)	0.540	-0.03 (-0.06- -0.003)	0.027	0.02 (-0.01-0.05)	0.237	0.04 (0.01-0.07)	0.003
Diastolic BP	0.0002 (-0.011-0.012)	0.966	0.002 (-0.009-0.013)	0.730	0.02 (0.01-0.03)	<0.001	0.01 (0.001-0.02)	0.027
Systolic BP	-0.003 (-0.008-0.003)	0.357	-0.002 (-0.007-0.004)	0.589	0.008 (0.002-0.01)	0.006	0.009 (0.003-0.015)	0.001
Age	-0.014 (-0.03- -0.001)	0.041	0.002 (-0.012-0.015)	0.823	0.007 (-0.006-0.02)	0.291	0.03 (0.02-0.04)	<0.001

	MMP9, beta (95%CI)	p	MMP10, beta (95%CI)	p	MMP12, beta (95%CI)	p
Sex	0.04 (-0.19-0.28)	0.726	0.13 (-0.12-0.37)	0.308	-0.057 (-0.30-0.18)	0.641
Diabetes	0.06 (-0.57-0.69)	0.843	0.43 (-1.14-0.27)	0.228	0.59 (-0.11-1.29)	0.100
Smoking	0.53 (0.31-0.75)	<0.001	0.55 (0.33-0.77)	<0.001	0.38 (0.16-0.60)	0.001
High alcohol consumption	0.08 (-0.45-0.62)	0.763	-0.13 (-0.71-0.45)	0.667	-0.18 (-0.76-0.40)	0.539
WBC	0.33 (0.28-0.38)	<0.001	0.14 (0.08-0.20)	<0.001	0.16 (0.10-0.22)	<0.001
BMI	0.012 (-0.015-0.040)	0.376	-0.18 (-0.046-0.010)	0.215	0.032 (0.004-0.060)	0.023
Diastolic BP	-0.0008 (-0.012-0.011)	0.881	0.002 (-0.009-0.013)	0.712	0.008 (-0.003-0.019)	0.160
Systolic BP	-0.002 (-0.008-0.003)	0.394	0.002 (-0.008-0.003)	0.415	0.007 (-0.001-0.012)	0.019
Age	-0.011 (-0.025-0.003)	0.114	-0.012 (-0.025-0.0023)	0.143	0.033 (0.020-0.046)	<0.001

WBC; white blood cell count, BMI; body mass index; BP, blood pressure.