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Matrix metalloproteinase-3 (MMP3) and MMP9 genes and risk of myocardial infarction, ischemic stroke, and hemorrhagic stroke

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Abstract

Objective—We assessed the association of genetic variation in MMP3 and MMP9 with risk of myocardial infarction and stroke.

Methods—A case-control study was conducted among members of Group Health (GH), a large integrated health care delivery system. Case subjects with incident non-fatal myocardial infarction (n = 854), ischemic stroke (n = 367), and hemorrhagic stroke (n = 66) were identified and validated. A matched control group was selected from among GH members without myocardial infarction or stroke (n=2696). Haplotype-tagging sets of single nucleotide polymorphisms (SNPs) in MMP3 and MMP9 were genotyped.

Results—MMP3 haplotype 2 was associated with reduced risk of myocardial infarction (adjusted odds ratio [OR] per copy = 0.80, 95% confidence interval 0.66, 0.98) and increased risk of hemorrhagic stroke (OR = 1.69, 95% confidence interval 1.05, 2.75). Results for MMP3 haplotype 2 and ischemic stroke resembled those for myocardial infarction but did not achieve statistical significance (OR = 0.85, 95% confidence interval 0.64, 1.12). No individual SNP identified MMP3 haplotype 2, and none of the individual MMP3 SNPs were associated with myocardial infarction or stroke. MMP9 haplotypes or SNPs were not associated with myocardial infarction or stroke.

Conclusions—MMP3 haplotype may predict both cardiac events and stroke.

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Keywords

Myocardial infarction; stroke; genetics

Matrix metalloproteinases (MMPs) are a family of more than 20 zinc-dependent proteinases with proteolytic activity against extracellular matrix (ECM) components including collagen and elastin, as well as numerous proteins involved in angiogenesis, cell migration, growth and apoptosis [1]. Each MMP has distinct although overlapping substrate specificities. Previous studies have suggested that MMPs may play a role in cardiovascular and cerebrovascular diseases. Human atheroma specimens display increased MMP-3 and MMP-9 expression in plaque shoulder regions and regions of foam cell accumulation [2]. MMP-2 is constitutively expressed in the central nervous system (CNS), localized in astrocytic processes in proximity to blood vessels, ependymal, and pial cells [3]. Expression of other MMPs including MMP-3 and MMP-9 in the brain and cerebral vasculature is low in the basal state, but may be upregulated in inflammatory responses [3].

Inter-individual variation in expression and activity of MMP3 and MMP9 appears to have a substantial genetic component. The population distribution of plasma MMP-9 levels has a bimodal distribution, suggesting the existence of distinct genotypic classes [1]. The 5A/6A polymorphism in the MMP3 promoter region (−1612) is a widely-studied gene locus. The 6A allele has been associated with lower promoter activity [4,5] and in turn, with reduced risk of cardiovascular disease in several studies [5–9]. Relatively few studies to date have examined haplotypic variation in MMP genes in relation to risk of cardiovascular and cerebrovascular disease [7,10].

In the present study, we examined whether common genetic variation in MMP3 and MMP9 predicted risk of incident myocardial infarction, ischemic stroke, and hemorrhagic stroke in a population-based, case-control study.

METHODS

Setting

The setting for this case-control study was Group Health (GH), an integrated health care delivery system based in Seattle, Washington, with over 500,000 members. The present analyses are part of a larger study based at GH of the pharmacogenetic epidemiology of cardiovascular events [11–13]. This study was approved by the GH Human Subjects Review Committee, and all study participants provided written informed consent.

Subjects

Case subjects were GH members who sustained a first hospitalized non-fatal myocardial infarction or non-fatal stroke during 1995 through 2002. We identified potential cases using computerized GH hospital discharges and confirmed events through medical records review [11]. Myocardial infarction events were confirmed by reviewing symptoms, cardiac enzymes, and electrocardiographic findings. Ischemic stroke events were confirmed according to the presence of a rapid-onset neurologic deficit that persisted at least 24 hours or evidence of infarction on brain imaging studies or at surgery. Hemorrhagic strokes were defined according to evidence of blood in the subarachnoid space or ventricles or dense intraparenchymal blood on brain imaging studies. Control subjects were a random sample of GH members, frequency-matched to the myocardial infarction case group (the largest case group) by decade of age, sex, menopausal status, the presence of treated hypertension, and study year at a ratio of at least 2:1. Cases and controls were eligible only if the medical records indicated that (1) they had not sustained a prior myocardial infarction or stroke, (2)

they were between the ages of 30 and 79, and (3) they had made at least 4 visits to a GH provider. Individuals identified as case or control subjects who were willing and able to provide a venous blood sample for genotyping were included in the present genetic study. Because of the design of the parent study, subjects included in analyses were hypertensive men, and postmenopausal women with and without hypertension.

Data collection

Each subject was assigned a reference date. The reference date for cases was the date of the myocardial infarction or stroke, and for controls was a randomly-selected date during the year for which they were selected as a control. Clinical information was collected using the medical records for the period prior to (but not including) the reference date. Thus, for cases, all clinical information preceded the occurrence of myocardial infarction or stroke. Trained abstractors recorded information on clinical cardiovascular risk factors including angina, diabetes, smoking, hypertension, blood pressure levels, height, weight, chronic obstructive pulmonary disease (COPD) and asthma, and recent laboratory values including total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and serum creatinine. Medical record abstractors were not blinded to case-control status, although they were unaware of the study hypotheses, and data collection was performed using comparable methods for cases and controls.

Selection and analysis of haplotypes

MMP3 and MMP9 were sequenced by the Seattle Program for Genomic Applications using DNA obtained from a panel of 24 Black/African-American and 23 White/European-American individuals, as previously described [12]. Haplotype-tagging sets of single-nucleotide polymorphisms (SNPs) were selected to describe genetic variation in Black/African-American and White/European-American individuals. These variants were then genotyped using the Illumina platform in the GH study population. This high-throughput genotyping platform only detects bi-allelic variants, and the aforementioned MMP3 promoter 5A/6A insertion/deletion was therefore not genotyped. One of the SNPs that was included (MMP3 SNP 12243 [*rs3025058J*]) is in tight linkage disequilibrium with this locus ($r^2 = 0.99$), so that this SNP provided information about the association of the MMP3 promoter insertion/deletion with study outcomes. Phased haplotypes were estimated using the genotype information and PHASE 2.0 software [14]. Haplotypes that had a frequency among controls below 5% were combined into a “rare haplotypes” category.

Statistical analysis

Characteristics of case and control subjects were compared using χ^2 and t-tests. Logistic regression with robust standard errors was performed to assess the association between MMP3 and MMP9 haplotypes and SNPs with risk of myocardial infarction, ischemic stroke, and hemorrhagic stroke, after adjustment for age, sex, and race/ethnicity. For global tests of case-control differences in the distribution of MMP3 and MMP9 haplotypes, we modeled all observed haplotypes as independent indicator variables and tested for overall statistical significance using the Wald test for the set of indicator variables. Ambiguous haplotypes were resolved by probability weighting [15], and the alternate approach of selecting the most probable haplotype was found to produce nearly identical results. For analyses of individual haplotypes and SNPs, we modeled risks according to a log-additive model for trend across 0, 1, and 2 haplotype or allele copies. Adjusted odds ratios (ORs) associated with each additional copy were estimated. Further analyses examined the effect of adjusting for established vascular risk factors including hypertension/blood pressure, hyperlipidemia, diabetes, and smoking. Results were repeated after stratification according to ancestry (self-reported race). Statistical analyses were performed using Stata 8.0.

RESULTS

Included were 854 individuals with myocardial infarction, 367 with ischemic stroke, 66 with hemorrhagic stroke, and 2,696 control subjects free of prior myocardial infarction and stroke (Table 1).

MMP3 gene

MMP3 is 13,003 bp in length with 10 exons. Sequencing was performed on 91.5% of the MMP3 gene (exon 3 was omitted), and 8 of 9 haplotype-tagging SNPs that were identified were successfully genotyped on case and control subjects. Global statistical testing indicated that overall genetic variation in MMP3 was not significantly associated with risk of myocardial infarction or stroke (Table 2). Further analyses identified significant associations between MMP3 haplotype 2 and risk of events. With non-carriers of haplotype 2 as the reference group, the adjusted OR per copy of haplotype 2 was 0.80 (95% confidence interval 0.66, 0.98) for myocardial infarction and 1.69 (95% confidence interval 1.05, 2.75) for hemorrhagic stroke. As for myocardial infarction, analyses of ischemic stroke suggested decreased risk with MMP3 haplotype 2, but the OR was not significantly different from the null (adjusted OR = 0.85, 95% confidence interval 0.64, 1.12). Results for the combined endpoint of myocardial infarction-plus-ischemic stroke were statistically significant (adjusted OR = 0.82, 95% confidence interval 0.69, 0.97). After additional adjustment for hypertension, systolic blood pressure, hyperlipidemia, diabetes, and smoking, the observed associations were similar although confidence intervals were wider (adjusted OR [95% confidence interval] = 0.83 [0.68, 1.02] for myocardial infarction, = 0.89 [0.67, 1.18] for ischemic stroke, and = 1.67 [1.03, 2.71] for hemorrhagic stroke). Associations between MMP3 haplotype 2 and outcomes persisted when analyses were limited to White/European-origin subjects. Other race/ethnic groups were too small to permit separate analyses. Analyses of individual MMP3 SNPs did not suggest a significant association between polymorphism at any single MMP3 locus and risk of myocardial infarction or stroke.

MMP9 gene

MMP9 is 11,224 bp in length with 13 exons. Sequencing was performed on 98.4% of the MMP9 gene (portions of exons 11 and 12 were omitted). For MMP9, 6 haplotype-tagging SNPs were identified (3 were non-synonymous) and 4 were successfully genotyped on cases and controls. MMP9 haplotypes and SNPs were not significantly associated with myocardial infarction or stroke (Table 3).

DISCUSSION

We observed that haplotypic variation in MMP3 (stromelysin-1) appears to be associated with vascular risk. Each additional copy of MMP3 haplotype 2 was associated with a statistically significant 20% lower risk of myocardial infarction when compared with non-carriers of this haplotype. Results for ischemic stroke were similar but did not achieve significance, which may reflect lower statistical power due to the smaller number of strokes as compared with the number of myocardial infarctions. In addition, each additional copy of MMP3 haplotype 2 was significantly associated with a 70% increase in the risk of hemorrhagic stroke. No single SNP unambiguously identified MMP3 haplotype 2, and none of the individual MMP3 SNPs was significantly associated with risk (Table 2). Our results suggest the presence of a MMP3 haplotype association in the absence of an MMP3 SNP association, and also suggest pleiotropic effects of MMP3 on cardiac and cerebrovascular endpoints. In contrast to results for MMP3, risk of vascular events was not associated with MMP9 haplotypes or SNPs.

This study is in agreement with several prior investigations indicating that genetic variation in MMP3 may affect risk of atherothrombotic events. One of the SNPs studied, MMP3 SNP 12243 (*rs3025058*), is in tight linkage disequilibrium ($r^2 = 0.99$) with the well-studied 5A/6A insertion/deletion at position -1612 in the MMP3 promoter. MMP3 haplotype 2, which appeared to protect against myocardial infarction (and predispose to hemorrhagic stroke), harbors the C allele in MMP3 SNP 12243, which is a marker for the MMP3 -1612 6A allele. Prior studies have linked the MMP3 promoter 6A allele with lower risk of acute coronary events [5–7] and ischemic stroke [16]. The 6A allele is also associated with reduced *in vitro* promoter activity and MMP-3 expression [4,5,17]. These data support the hypothesis that activity of MMP-3, which is present in atherosclerotic lesions [2], may increase the risk of acute vascular events by mediating inflammation or plaque rupture [2,3,18]. We also identified an association between MMP3 haplotype 2 and increased risk of hemorrhagic stroke. Matrix-degrading enzyme activity may play a role in the etiology of brain hemorrhage by disrupting the blood-brain barrier or favoring the development of aneurysms, consistent with studies that have altered MMPs and tissue inhibitors of MMPs (TIMPs) in animal models [19,20]. Prior epidemiological studies of MMP3 and other MMP genes in relation to risk of hemorrhagic stroke [21,22] and intracranial aneurysm [23] have been null, but data in this area are incomplete and prior studies have been limited to single SNPs. Our finding that the same MMP3 haplotype appeared to protect against thrombotic events (ie, myocardial infarction) and promote bleeding events (ie, hemorrhagic stroke) may also be explained by cleavage of coagulation and fibrinolytic proteins (plasminogen, plasminogen activator inhibitor-1, fibrinogen) by MMP-3 [24–27].

Our study did not show any significant association between genetic variation in MMP9 and vascular risk. One of the variants studied, MMP9 SNP 7556, is in tight linkage ($r^2 = 0.99$) with a previously-identified variant in the gene promoter region (MMP9 C-1562T) that has been reported to predict MMP expression and vascular disease [28].

Exclusion of nonfatal cases is a limitation of this study. Because relatively few individuals were of non-White ethnicity, our ability to draw conclusions about groups with other ancestral backgrounds is limited. In addition, our findings will require replication in future studies.

In summary, this study identified a haplotype in MMP3 that predicted decreased risk of myocardial infarction and increased risk of hemorrhagic stroke. A notable finding was the demonstration of a haplotype effect of MMP3 on vascular risk that is not likely to be fully explained by MMP3 -1612 5A/6A or any other previously-identified risk alleles [5–7,16]. Thus, the use of haplotype-tagging SNP sets in this study to describe genetic variation in MMP3 appeared to be important. This finding further supports prior evidence that genetic variation in MMP3 may contribute to risk of vascular disease.

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Table 1

Characteristics of Group Health members with incident myocardial infarction, ischemic stroke, and hemorrhagic stroke, and of control subjects.

	Control n = 2696			Myocardial infarction n = 854			Ischemic stroke n = 367			Hemorrhagic stroke n = 66		
	Mean	SD		Mean	SD	p vs control	Mean	SD	p vs control	Mean	SD	p vs control
Age, years*	65.3	9.8		65.8	9.8		68.5	9.1		65.8	9.5	
Systolic BP, mm Hg	138.2	18.6		142.2	20.2	<0.001	146.2	22.1	<0.001	144.1	23.1	0.0117
Diastolic BP, mm Hg	80.5	10.6		80.5	11.4	0.8993	81.6	11.7	0.0778	83.8	14.0	0.0142
Body mass index, kg/m ²	29.5	6.3		30.1	6.1	0.0191	30.1	6.9	0.1342	28.7	6.7	0.2932
Total cholesterol, mg/dl	221.2	54.4		231.5	47.8	<0.001	229.3	44.2	0.0068	223.6	48.2	0.7229
Serum creatinine, mg/dl	1.0	0.4		1.1	0.7	<0.001	1.1	0.7	0.0045	1.1	0.9	0.3564
HDL cholesterol, mg/dl	53.3	17.0		49.0	15.4	<0.001	51.7	17.0	0.0925	55.7	16.5	0.2735
LDL cholesterol, mg/dl	130.4	37.6		138.8	43.8	<0.001	137.0	36.5	0.0237	136.9	47.0	0.368
	N	Percent		N	Percent	p vs control	N	Percent	p vs control	N	Percent	p vs control
Female*	1,572	58.3		489	57.3		253	68.9		48	72.7	
White/European-American	2,457	91.1		778	91.1	0.999	334	91.0	0.059	57	86.4	0.336
Black/African-American	100	3.7		32	3.8		21	5.7		3	4.6	
Other race	139	5.2		44	5.2		12	3.3		6	9.1	
Angina	209	7.8		144	16.9	<0.001	33	9.0	0.41	1	1.5	0.059
Hypertension*	1,978	73.4		614	71.9		263	71.7		41	62.1	
Diabetes	305	11.3		207	24.2	<0.001	90	24.5	<0.001	7	10.6	0.858
Hyperlipidemia	345	12.8		142	16.7	0.004	45	12.3	0.773	5	7.6	0.208
Aspirin use	1,446	60.3		400	52.7	<0.001	167	51.4	0.002	32	56.1	0.523
COPD/Asthma	322	11.9		136	15.9	0.002	64	17.4	0.003	12	18.2	0.125
Current smoking	270	10.0		155	18.2	<0.001	46	12.5	<0.001	12	18.2	0.045
Former smoking	1,151	42.7		362	42.4		123	33.5		17	25.8	

* Age, sex, and treated hypertension status were matching variables.

BP, blood pressure; COPD, chronic pulmonary obstructive disease

[†] Global test performed as joint test for differences across all haplotypes (5 degree of freedom χ^2 test) Minor allele is indicated in **bold**

SNP, single nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; Eur., European descent; Afr., African descent

Table 3

Association between haplotypes and SNPs in MMP9 and risk of myocardial infarction and stroke.

		Locus: Seattle SNPs ID (rs #)				Haplotype associations			
		4670 (rs2664538)	6851 (rs2250889)	7556 (rs2274756)	8215 (rs3918262)		Myocardial infarction	Ischemic stroke	Hemorrhagic stroke
						Freq.	95% CI	95% CI	95% CI
Haplotype #	MAF								
1	Eur	0.42	0.09	0.14	A	0.60	1.00 0.89, 1.11	1.05 0.89, 1.23	0.85 0.61, 1.19
2	Afr	0.41	0.13	0.23	A	0.05	0.93 0.74, 1.18	1.18 0.87, 1.61	0.74 0.35, 1.58
3		G	C	G	G	0.20	1.04 0.91, 1.19	0.90 0.74, 1.10	1.34 0.91, 1.98
4		G	C	A	A	0.14	0.99 0.85, 1.17	0.96 0.76, 1.22	1.02 0.64, 1.63
Rare haplotypes						0.01	0.81 0.47, 1.42	1.04 0.51, 2.09	0.92 0.25, 3.31
Global test for haplotype effect [†]									
p = 0.88									
p = 0.70									
p = 0.60									
-----SNP Associations-----									
Myocardial infarction									
OR*		1.02	1.09	1.01	1.04				
95% CI		0.9, 1.1	0.9, 1.4	0.9, 1.2	0.9, 1.2				
Ischemic stroke									
OR*		0.91	0.82	1.04	0.91				
95% CI		0.8, 1.1	0.6, 1.1	0.8, 1.3	0.7, 1.1				
Hemorrhagic stroke									
OR*		1.27	1.17	0.98	1.34				
95% CI		0.9, 1.8	0.6, 2.4	0.6, 1.6	0.9, 2.0				

* Odds ratio for trend across 0,1,2 copies of haplotype or allele, adjusted for age, sex, and race/ethnicity.

[†] Global test performed as joint test for differences across all haplotypes (4 degree of freedom χ^2 test) Minor allele is indicated in **bold** SNP, single nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; Eur., European descent; Afr., African descent