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Original article

Gender-specific effects of *MMP-2* and *MMP-9* gene variants and the risk of aneurysmal subarachnoid haemorrhage

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ABSTRACT

Matrix metalloproteinases (MMPs), a family of zinc-dependent proteases, have been linked with the pathogenesis of intracranial aneurysm (IA) formation and rupture. Patients with IA have elevated serum levels of MMP-2 and *MMP-9* gene expression has been shown to be increased in aneurysm tissue. In this study, we have evaluated the association between *MMP* gene variants and aneurysmal subarachnoid hemorrhage (aSAH). The rs243865 in *MMP-2* and rs17576 in *MMP-9* genes were genotyped using Taqman allelic discrimination assay. Upon gender stratification, the presence of G allele of *MMP-9* gene exon variant was found to increase the risk of aSAH by 1.4 fold ($p = 0.032$), in men. The GG genotype also showed a trend towards association with the risk of aSAH (OR: 1.785, CI: 0.971–3.283, $p = 0.062$) in men, after adjusting for the vascular risk factors. In women, no association was observed. The *MMP-2* gene variants were not associated with aSAH in both genders. Further functional studies are required to describe the exact basis of the association between *MMP-9* polymorphism and intracranial aneurysm in men.

1. Introduction

Subarachnoid hemorrhage due to the rupture of an intracranial aneurysm is a catastrophic cerebrovascular accident with high rate of mortality and morbidity (Zacharia et al., 2010). The etio-pathogenesis of aneurysmal subarachnoid hemorrhage (aSAH) is unclear with diverse factors playing roles. Seven to 20% of aSAH patients have first or second degree relatives with intracranial aneurysm (IA) and familial IA rupture occurs 5–10 years earlier compared to non familial rupture. This indicates that genetic factors are likely to play a crucial role in IA formation and rupture beside environmental causes (Mensing et al., 2016; Wang et al., 2002).

The major pathomechanism involved in aneurysm formation is speculated to be chronic inflammation due to hemodynamic stress at the bifurcations of blood vessels (Hashimoto et al., 2006). Infiltrating macrophages and neutrophils secrete matrix metalloproteinases (MMPs), which act upon extracellular matrix (ECM) proteins leading to vessel wall degeneration and aneurysm development (Aoki et al., 2007). MMPs are zinc-dependent proteolytic enzymes that degrade ECM proteins such as elastin, collagens, gelatins and proteoglycans which are involved in the maintenance of vascular integrity (Maradni et al., 2013). Histopathological studies of IAs have revealed complete or partial degradation of the internal elastic lamina of the vessel wall by MMPs.

Genetic variations in the *MMP* genes may affect their expression at the level of transcription or enzyme activity (Krex et al., 2004). The role *MMP* gene variants in the pathogenesis of IA have not been previously studied in Asian Indian population and hence, in this report we investigated the association of *MMP-2* and *MMP-9* gene variants with susceptibility to aSAH.

2. Methodology

2.1. Study population

The study was conducted at the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, India. Ethical clearance was obtained from the Institutional Ethics Committee and informed consents were taken from all the subjects for their participation in the study. Two hundred and seventy one cases admitted to the

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Neurosurgery casualty unit and diagnosed with aneurysmal SAH were recruited. Diagnosis of aSAH was based on clinical history and neuroimaging studies (CT scan head, CT angiography and digital subtraction angiography). The severity of SAH was scaled according to World Federation of Neurosurgical Societies (WFNS) grading system and at the time of discharge, the outcome was assessed by Glasgow outcome scale (GOS). Two hundred and eighty clinically normal, age- and gender-matched volunteers were recruited as controls.

2.2. Blood sample collection and genotyping

Five mL of venous blood was collected from all subjects and the genomic DNA was isolated by conventional phenol-chloroform method (Sambrook et al., 1989). The DNA was quantified using NanoDrop 2000 (ThermoFisher Scientific, MA, USA). Based on the previous and recent studies that support the role of *MMP* gene variants in IA formation and rupture, rs243865 SNP in *MMP-2* (Alg et al., 2018 and Low et al., 2011) and rs17576 in *MMP-9* (Wang et al., 2018 and Pannu et al., 2006) genes were selected and genotyped using Taqman allelic discrimination assay. The fluorescent probes C_11655953_10 for rs17576 and C_3225943_10 for rs243865 (Applied Biosystems, Foster City, CA, USA) were used for allelic discrimination. Polymerase chain reaction was carried out in a volume of 10uL reaction (comprising of 2× Taqman genotyping master mix, 40× assay probes and 5 ng of DNA per well in a 96-well plate) in a real time thermo cycler (Applied Biosystems 7500 Fast, Foster City, CA, USA) under the following conditions: enzyme activation at 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s and annealing/extension at 60 °C for 1 min.

2.3. Statistical analysis

Demographic parameters were compared between cases and controls using *t*-test for continuous parameters and chi-squared test for categorical variables. The genotype and allele frequencies were compared among different groups using chi-squared test. Genotypes and alleles followed Hardy-Weinberg equilibrium in the study population. Univariate odds ratios (ORs) as an estimate of risk was performed and multiple logistic regression analysis was used to adjust ORs for covariates. To identify gender-specific association of the gene variants with IA development, stratified analysis between genders was performed, which compared the OR of the associated SNP between the two genders. $P < 0.05$ was considered statistically significant. All the statistical analyses were carried out using SPSS v22.0 statistical software.

3. Results

The mean age of aSAH patients and healthy controls was 50.63 years (129 men and 142 women), and 49.37 years (145 men and 135 women), respectively. Patients differed significantly from controls with respect to smoking (30% vs. 13%, p = 0.0001), alcohol consumption (28% vs. 11.8%, p = 0.0001) and hypertension (28% vs. 10.7%, p = 0.0001). The clinical features of aSAH patients were headache (97.2%), vomiting (98%), seizures (18.1%), altered sensorium (55%), and focal deficits (27.61%). Eighty one percent underwent clipping and 11% coiling for the ruptures aneurysms. There was no significant difference in genotype frequencies between aSAH cases and controls. However the presence of "G" allele of *MMP-9* variant was found to confer 1.4-fold increased risk (CI: 1.047–2.058, p = 0.032, Table 1) of aSAH in men. Under different models of inheritance, recessive model (GG vs. AA + AG) of *MMP-9* variant was associated with a 1.6-fold (CI: 0.996–2.847, p = 0.050, Table 2) increase in odds for aSAH in men. However, the significance was reduced after adjusting with covariates such as hypertension, smoking and alcohol intake. We did not detect any association between *MMP-2* gene variant and aSAH in either men or women.

Cases were divided into different subgroups based on aneurysm size, WFNS grade and outcome to check whether these *MMP* gene variants have any effect in aSAH patients (Table 3). However, the distribution of genotypes did not vary significantly among subgroups and hence no association was observed.

Table 1
Allelic frequency of *MMP 2* and *MMP 9* gene variants in males.

Genotype	Allelic frequency				OR (95% CI)	P Value
	Controls		Cases			
MMP 2 (rs243865)	C 0.872	T 0.127	C 0.848	T 0.151	1.220(0.749–1.983) 0.820(0.504–0.334)	0.461
MMP 9 (rs17576)	A 0.418	G 0.581	A 0.513	G 0.486	0.681(0.485–0.955) 1.468(1.047–2.058)	0.032*

* p values < 0.05 is statistically significant.

4. Discussion

Experimental studies have demonstrated that patients with aSAH have elevated serum levels of MMP-2 (Todor et al., 1998 and Rojas et al., 2018) and over expression of MMP-2 (Bruno et al., 1998), MMP-9 (Todar et al., 1998, Jin et al., 2007) in human cerebral aneurysmal tissues when compared with control samples. Also, it is reported that serum MMP-9 level was positively correlated with common carotid artery intima media thickness in patients with ischemic stroke and may be associated with atherogenesis (Abdelnaseer et al., 2016). Increased MMP activity would further lead to atherogenesis and apoptosis in the vascular vessel wall ultimately causing aneurysm formation and rupture (Caird et al., 2006).

The regulation of MMPs occurs at different levels of transcription and translation (Vandooren et al., 2013). Therefore, variants in the *MMP* genes might influence the transcriptional activity or gene expression and give rise to a missense or nonsense mutation (Krex et al., 2004; Katarkar et al., 2018). Consequently, this could have an effect on the functional characteristics of the MMPs. Association of *MMP* gene variants have been implicated in coronary heart diseases (Wu et al., 2013 and Mishra et al., 2012), chronic obstructive pulmonary diseases (Haq et al., 2010), various cancers (Zhou et al., 2011 and Brooks et al., 2010) and abdominal aortic aneurysm formation (Li et al., 2018). Hence in this report, we have investigated the association between *MMP-2* and *-9* gene variants and the risk of aSAH.

The rs17576 in the *MMP-9* gene is present on chromosome 20, exon 6, A > G. It is also known as Gln279Arg or Q279R. The rs243865 of *MMP-2* variant is located on chromosome 16, and it is an intronic variant C > T. Even though rs243865 is present in the intron region, it appears to be a functional variant. The C > T transition has been shown to abolish the binding site for the transcription factor-specificity protein (Sp1) which in turn may affect the expression and activity of MMP-2. By recruiting and combining with other transcription factors, Sp1 may either act as transcription activator or repressor (Price et al., 2001; Alg et al., 2018). Hence, these *MMP* gene variants may be important markers to test for an association in IAs.

Based on gender-stratified analysis, we found that the *MMP-9* gene variant (rs17576) in the exon-6 showed a trend towards significance for increased odds for aSAH in men but not in women. This could be due to the protective effects of estrogen against MMPs in women. Tetyana and coworkers (Tetyana and Jason, 2010) have shown that estrogen treatment improved TIMP-2/MMP-2 and TIMP-1/MMP-9 protein balance, restored ER alpha expression, and prevented MMP-9 activation, perivascular collagen accumulation and development of heart failure in ovariectomized female rats. In experimentally-induced cerebral aneurysm rat models, the expression of MMPs increased with the progression of aneurysm (Aoki et al., 2007). Hence, to check the effect of